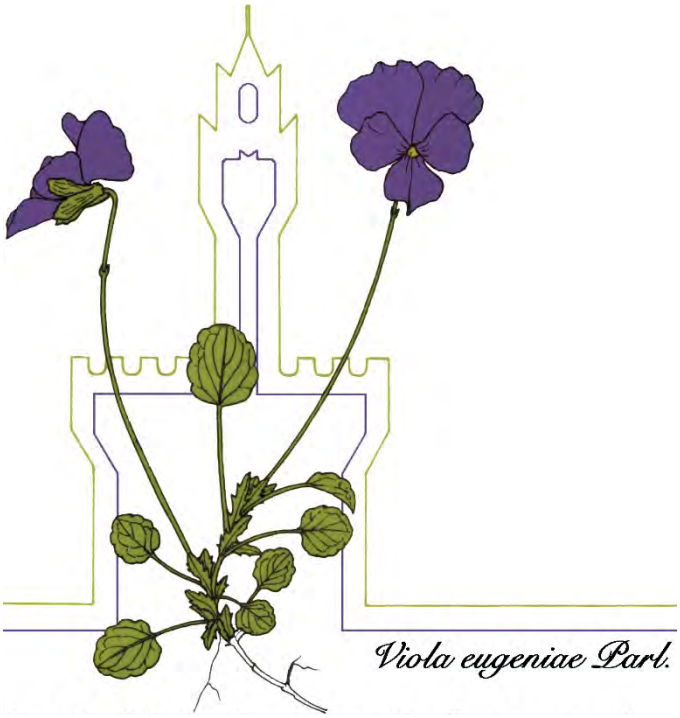
# 118° Congresso della Società Botanica Italiana

## IX INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC)

## PISA, 13 - 16 SEPTEMBER 2023




# ABSTRACTS

## KEYNOTE LECTURES, COMMUNICATIONS, POSTERS

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# 118° Congresso della Società Botanica Italiana onlus

IX INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC)

Pisa, Polo Didattico San Rossore 1938, 13 - 16 September 2023

## Programme

## Wednesday 13 September 2023

9:30-12:30 Administrative Meetings of the Working Groups. Registration

13:00-13:45 **Opening ceremony**

13:45-14:15 Plenary opening lecture: **Jennifer McElwain**, Trinity College Dublin, Ireland (25 + 5 min)
"A journey through Earth's deep past: 400 million years of plant and atmospheric evolution"

### Symposium 1

**MOLECULAR AND CELL BIOLOGY**
(Chairpersons: L. Navazio and L. Sanità di Toppi)

**Key words:** Algae, Bryophytes, Functional and integrative biology, Fungi, Lichens, Stresses

14:30-15:00
- Invited lecture: **Vasileios Fotopoulos**, Cyprus University of Technology, Cyprus (25 + 5 min)
"The role of reactive oxygen, nitrogen and sulfur species as regulators of plant abiotic stress responses"

15:00-16:30 **Communications**

- Federica Brandizzi, **Luciana Renna** (12 + 3 min)
"Multiple contact sites investigation for chloroplast homeostasis in plant cells"
- **Claudio Varotto**, Enrico Barbaro, Daniela Bertoldi et al. (12 + 3 min)
"*ACR3* is a key player in arsenic detoxification in bryophytes"
- Mara Cucinotta, Alex Cavalleri, **Marta A. Mendes** et al. (12 + 3 min)
"Ovule number and fertility are regulated epigenetically during drought stress conditions"
- Eleonora Greco, **Emanuela Talarico**, Maria Letizia Madeo et al. (12 + 3 min)
"Short term exposition to acute cadmium toxicity induces the loss of root gravitropic stimuli perception through PIN2 polar auxin transport in *Arabidopsis thaliana*"
- **Silvia Perotto**, Silvia De Rose, Yukari Kuga et al. (12 + 3 min)
"Nutrient transfer and metabolism in orchid mycorrhiza, as revealed by gene expression and stable isotope labelling in the *Serapias vomeracea-Tulasnella calospora* association"
- **Luigi Sanità di Toppi** (12 + 3 min)
"From *Marchantia polymorpha* to *Arabidopsis thaliana*: old and new roles of the 'multi-tasking' enzyme phytochelatin synthase in land plants"

16:30-17:00 Coffee Break

### Symposium 2

**TAXONOMY, SYSTEMATICS AND EVOLUTION**
(Chairpersons: F. Selvi and S. Tosi)

**Key words:** Algae, Bryophytes, Fungi, Lichens, Tropical plants, Vascular plants

17:00-17:30
- Invited lecture: **Christoph Oberprieler**, University of Regensburg, Germany (25 + 5 min)
"Wettstein goes Carrara: integrative species delimitation in hybridising plant groups"

17:30-19:00 **Communications**

- **Martina Braconcini**, Susanna Gorrasi, Paolo Barghini et al. (12 + 3 min)
  "Diversity of marine fungi associated with the ascidian *Halocynthia papillosa*"
- **Beatriz Lucas Arida**, André V. Lucci Freitas, Maria G. Gutierrez Camargo et al. (12 + 3 min)
  "What is the role of pollinator-mediated selection on the floral coloration polymorphism of the Neotropical orchid *Epidendrum fulgens*?"
- **Martino Adamo**, Katarina Skokanová, Javier Bobo-Pinilla et al. (12 + 3 min)
  "Molecular evidence and environmental niche evolution at the origin of the disjunct distribution in three mountain endemic *Tephroseris* (Asteraceae) of the Mediterranean basin"
- **Domenico Gargano**, Hirzi Luqman, Domenico Amantea et al. (12 + 3 min)
  "Experimental crosses suggest multifaceted patterns of reproductive barriers among genetically distinct lineages of the *Dianthus virgineus* complex (Caryophyllaceae)"
- **Manuel Tiburtini**, Silvia Fruzzetti, Liliana Bernardo et al. (12 + 3 min)
  "A morphometric study of Calabrian-Sicilian Endemic *Armeria* taxa (Plumbaginaceae)"
- **Gianniantonio Domina**, Daniela Longo, Eckhard von Raab-Straube et al. (12 + 3 min)
  "A revision of *Oncostema* (Asparagaceae, Scilloideae) in Italy"

19:30-21:00 **Welcome cocktail at the Botanic Garden and Museum of the University of Pisa**

## Thursday 14 September 2023

### Symposium 3

**BIODIVERSITY**

(Chairpersons: A. Florenzano and M. Nepi)

**Key words:** Archaeobotany, Biodiversity informatics, Diversity in reproductive strategies, Field and remote monitoring, Floristics, Palynology, Plant conservation, Research in Botanical Gardens

8:30-10:30 **Communications**

- **Assunta Florenzano** (12 + 3 min)
  "Archaeobotany to explore long-term environmental changes and human impact in Italy"
- **Gianni Bedini**, Fabrizio Bartolucci, Fabio Conti et al. (12 + 3 min)
  "Floritaly, an R package to access the Checklists of the vascular flora of Italy"
- Alessio Mo, **Diana Cruz**, Alice Fuggirai et al. (12 + 3 min)
  "Seed germination responses of annual back dune species to current and future climatic scenarios"
- **Andrea Bertacchi**, Tiziana Lombardi (12 + 3 min)
  "UAV monitoring of the spread of *Juniperus macrocarpa* on the dunes of NW Tuscany"
- **Luana Francesconi**, Matteo Conti, Gabriele Gheza et al. (12 + 3 min)
  "Dolichens project: a dynamic inventory of the lichen biota of the Dolomites"
- **Cristiano Vignola**, Alessia Masi, Jordan Palli et al. (12 + 3 min)
  "Palynological evidence of climatic variability and reforestation in Italy during the Little Ice Age (LIA)"
- **Angelino Carta**, Simone Orsenigo, Elena Zappa et al. (12 + 3 min)
  "Phylogenetic and trait-based correlates of extinction risk in Italian flowering plants"
- **Marco Canella**, Núria Beltrán-Sanz, Valentina Boscariol et al. (12 + 3 min)
  "Alpine Botanic Gardens as treasure chests of biodiversity for the quasi in situ conservation of Plant Genetic Resources"

10:30-11:00 Coffee Break

### Symposium 4

**ENVIRONMENTAL MONITORING AND POLICIES**

(Chairpersons: S. Loppi and A. Montagnoli)

**Key words:** Bioindicators, Biological invasions, CITES, Conservation policies, Forestry, Monumental trees, Phytoremediation, Plants and environment

11:00-13:00 **Communications**

- **Lisa Grifoni**, Aldo Winkler, Francesca Boldrighini et al. (12 + 3 min)
  "Magnetic and chemical biomonitoring with lichens and trees leaves for the conservation of cultural heritage"
- **Lara Assunta Quaglini**, Florencia Yannelli, Federica Fasano et al. (12 + 3 min)
  "Influence of the structure of resident plant communities and abiotic characteristics on the performance of the invasive alien species *Senecio inaequidens*"
- **Emanuele Pelella**, Flaminia Mariani, Simona Ceschin (12 + 3 min)
  "Impact of the invasive alien macrophyte *Ludwigia hexapetala* on freshwater ecosystems"
- **Viola Alessandrini**, Iduna Arduini (12 + 3 min)
  "Effects of the invasive alien *Salpichroa origanifolia* on the soil seed bank of a mixed forest (San Rossore, Migliarino, Massaciuccoli Regional Park, Pisa)"
- **Martina Pollastrini**, Filippo Bussotti (12 + 3 min)
  "Forest monitoring updates: physiological indicators of tree health for detecting and understanding a changing environment"
- **Annamaria Gentile**, Patrizia Iannece, Ivano Spiniello et al. (12 + 3 min)
  "Contaminants of emerging concern and antimicrobial resistance: use of nature-based solutions for a safe civil wastewater reus"
- **Alice Peduzzi**, Elisa Brasili, Diego Piacentini et al. (12 + 3 min)
  "Salt-stress, effects on the root system of two *Sorghum bicolor* hybrids: morpho-functional, cytohistological and metabolomic analyses"
- **Alba Cuena-Lombraña**, Mauro Fois, Michela Marignani et al. (12 + 3 min)
  "Threats and vulnerabilities of Sardinian wetlands: from environmental to botanical perspective"

13:00-14:00 Lunch

**PARALLEL SESSIONS OF ORAL POSTER PRESENTATIONS 1**

14:00-15:45 oral poster presentations (3+2 minutes) in 4 parallel sessions

Parallel session A1 (Chairpersons A. Andreucci, L. Sanità di Toppi): 4.8. Plants and environment; 5.4. Biotechnologies

Parallel session B1 (Chairpersons A. Carta, L. Peruzzi): 2.5. Tropical plants; 2.6. Vascular plants; 3.1. Archaeobotany; 3.2. Biodiversity informatics; 3.3. Diversity in reproductive strategies; 3.6. Palynology

Parallel session C1 (Chairpersons A. Braca, G. Flamini): 5.3. Bioactivities; 5.7. Medicinal plants; 5.8. Phytochemistry

Parallel session D1 (Chairpersons A. Bertacchi, D. Ciccarelli): 4.5. Forestry; 5.5. Cultural heritage; 6.2. Ecosystems and communities; 6.3. Functional traits

15:45-16:15 Coffee Break

**PARALLEL SESSIONS OF ORAL POSTER PRESENTATIONS 2**

16:15-17:45 oral poster presentations (3+2 minutes) in 4 parallel sessions

Parallel session A2 (Chairpersons L. Paoli, M. Ruffini Castiglione): 1.1. Algae; 1.2. Bryophytes; 1.3. Functional and integrative biology; 1.4. Fungi; 1.6. Stresses; 2.3. Fungi; 4.1. Bioindicators; 4.7. Phytoremediation

Parallel session B2 (Chairpersons G, Bedini, A. Carta): 3.5. Floristics; 3.7. Plant conservation; 3.8. Research in Botanical Gardens

Parallel session C2 (Chairpersons R. Ascrizzi, L. Pistelli): 5.1. Agrobiodiversity; 5.2. Applied symbiotic interactions; 5.6. Food plants; 6.5. Specialized metabolites

Parallel session D2 (Chairpersons I. Arduini, A. Bertacchi): 3.4. Field and remote monitoring; 4.2. Biological invasions; 6.1. Climate change; 6.6. Vegetation

18:00-19:00 **General Meeting of the Italian Botanical Society (members only)**

20:30-23:30 **Congress social dinner**

## Friday 15 September 2023

### Symposium 5

**BIOTECHNOLOGY AND APPLIED BOTANY**

(Chairpersons: N. De Tommasi and M. Lenucci)

**Key words:** Agrobiodiversity, Applied symbiotic interactions, Bioactivities, Biotechnologies, Cultural heritage, Food plants, Medicinal plants, Phytochemistry

8:30-10:30 **Communications**

- **Gemma Chiaffarelli**, Nicolò Sgalippa, Ilda Vagge (12 + 3 min)
  "Agrobiodiversity decline and recovery: a multi-scale issue. A pilot assessment experience in the Po Plain district"
- **Matteo Chialva**, Davide Lucien Patono, Leonardo Perez de Souza et al. (12 + 3 min)
  "The mycorrhizal root-shoot axis elicits *Coffea arabica* growth under low phosphate conditions"
- **Maria Ponticelli**, Vittorio Carlucci, Daniela Russo et al. (12 + 3 min)
  "Olive oil exhausted pomace Green extraction techniques: phytochemical characterization and biological activities"
- **Natasha Damiana Spadafora**, Antonella Muto, Carsten Muller et al. (12 + 3 min)
  "Simultaneous characterisation of *Prunus persica* fruit volatilome and gene expression profile for a better understanding of molecular mechanisms underlying changes of peach during post-harvest storage"
- **Giulia Caneva** (12 + 3 min)
  "Wonder, Power, and Love: Agostino Chigi's message written in the language of plants at the dawn of a new era"
- **Massimo Tacchini**, Gianni Sacchetti, Chiara Chiozzini et al. (12 + 3 min)
  "In vitro digestion of three Ethiopian varieties of *Eragrostis tef*"
- **Simona Trincia**, Ilaria Chiocchio, Manuela Mandrone et al. (12 + 3 min)
  "Metabolomic analysis and bioactivities of *Arbutus unedo* leaves harvested across the seasons in different natural habitats of Sardinia (Italy)"
- **Annunziata Paolillo**, Francesco Sottile, Milena Masullo et al. (12 + 3 min)
  "Phytochemical investigation of *Cornus sanguinea*"

10:30-11:00 Coffee Break

### Symposium 6

**ECOLOGY**

(Chairpersons: F. Antognoni and P. Fortini)

**Key words:** Climate change, Ecosystems and communities, Functional traits, Life strategies, Specialized metabolites, Vegetation

11:00-11:30
- Invited lecture, **Douglas L. Godbold**, University of Bodenkultur Wien, Austria (25 + 5 min)
  "Who's driving who? Community ecology of trees and ectomycorrhizas"

11:30-13:00 **Communications**

- **Silvano Lodetti**, Margherita Tognela, Pietro Fanchini et al. (12 + 3 min)
  "Multifaceted assessment of vegetation and soil temperatures changes in the Orobic Alps after 13 years of monitoring"
- **T'ai Gladys Whittingham Forte**, Michele Carbognani, Giorgio Chiari et al. (12 + 3 min)
  "Vegetation dynamics and species responses of an alpine grassland to 5 years of experimental warming and drought"
- **Nicodemo Giuseppe Passalacqua**, Simone Rovito, Antonio Lagudi (12 + 3 min)
  "Surveying pasture communities in diachronic analyses by 3D Models"
- **Daniela Ciccarelli**, Cleusa Bona, Angelino Carta (12 + 3 min)
  "Coordination between leaf and root traits in Mediterranean coastal dune plants"
- **Emily Cioni**, Alessandra Braca, Fabiano Camangi et al. (12 + 3 min)

"LC-MS untargeted metabolomic analysis of the halophyte *Salsola tragus* in the climate change scenario revealed a promising phytocomplex"

- **Cristina Gasperini**, Kurt Bollmann, Jörg Brunet et al. (12 + 3 min)
"Soil seed bank responses to edge effects in temperate European forests"

### Symposium 7

**A COSA SERVIRÀ MAI LA (STORIA DELLA) BOTANICA?**
(Moderatore G. Carrada)

14:00-16:30 Lo studio della Storia della Botanica non rappresenta un classico studio "antiquario", fine a sé stesso, bensì assume oggi un significato innovativo e rilevante, sia per chi fa ricerca, sia per illuminare aspetti importanti della nostra cultura. Quest'anno ricorrono i 2000 anni dalla nascita di Plinio il Vecchio, botanico antesignano, e i 480 anni dalla fondazione dell'Orto Botanico di Pisa. Assieme ad autorevoli rappresentanti di alcune delle sedi botaniche italiane più significative dal punto di vista storico, questo Simposio si propone di ricostruire vicende, storie e personaggi che hanno contribuito a tenere alto il nome della Botanica Italiana e, più in generale, della cultura del nostro Paese.

Interverranno:

**Barbara Baldan** (Università di Padova), **Paolo Caputo** (Università di Napoli "Federico II"), **Juri Nascimbene** (Università di Bologna), **Alessio Papini** (Università di Firenze), **Lorenzo Peruzzi** (Università di Pisa), **Fabio Attorre** (Sapienza Università di Roma), **Maria Consolata Siniscalco** (Università di Torino), **Francesco Maria Raimondo** (Università di Palermo), **Solveig Tosi** (Università di Pavia)

16:30-17:00 **Congress Closure**

## Saturday 16 September 2023

### Evento Post-Congress

9:00-13:00 Field excursion in the San Rossore Estate (Pisa), Natural Park of Migliarino - San Rossore – Massaciuccoli

*The timetable may be modified*

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# Communications

## SYMPOSIUM 1.
## "MOLECULAR AND CELL BIOLOGY"



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## "TAXONOMY, SYSTEMATICS AND EVOLUTION"



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## "BIODIVERSITY"

SYMPOSIUM 4.
"ENVIRONMENTAL MONITORING AND POLICIES"

SYMPOSIUM 5.
"BIOTECHNOLOGY AND APPLIED BOTANY"



SYMPOSIUM 6.
"ECOLOGY"

# Posters

SYMPOSIUM 1.
"MOLECULAR AND CELL BIOLOGY"

## 1.1 Algae



## 1.2 Bryophytes



## 1.3 Functional and integrative biology



## 1.4 Fungi



## 1.6 Stresses

### 3.3 Diversity in reproductive strategies



### 3.4 Field and remote monitoring



### 3.5 Floristics

### 3.8 Research in Botanical Gardens



## SYMPOSIUM 4.
## "ENVIRONMENTAL MONITORING AND POLICIES"

### 4.1 Bioindicators



### 4.2 Biological invasions

### 4.5 Forestry



### 4.7 Phytoremediation



### 4.8 Plants and environment

SYMPOSIUM 5.
"BIOTECHNOLOGY AND APPLIED BOTANY"

## 5.1 Agrobiodiversity

## 5.2 Applied symbiotic interactions



## 5.3 Bioactivities

## 5.4 Biotechnologies



## 5.5 Cultural heritage

## 5.6 Food plants



## 5.7 Medicinal plants

## 5.8 Phytochemistry



SYMPOSIUM 6.
"ECOLOGY"

## 6.1 Climate change

### 6.2 Ecosystems and communities



### 6.3 Functional traits



### 6.5 Specialized metabolites

## 6.6 Vegetation



Link to posters

https://drive.google.com/drive/folders/1L5XNT2jW-s90Fmf8pYfLVxyFD2Fl96Of?usp=sharing

# KEYNOTE LECTURES

## A journey through Earth's deep past: 400 million years of plant and atmospheric evolution

Jennifer McElwain
School of Natural Sciences, Discipline of Botany, Trinity College Dublin, Ireland

Climate change forced by rising level of atmospheric greenhouse gasses is now evident on every continent and every ecosystem. Atmospheric carbon dioxide levels are higher than any time in the past 15 million years based on new results from the $CO_2$ Proxy Integration Project consortium which include extensive proxy atmospheric $CO_2$ records based on ecophysiology models, stomatal and stable carbon isotopic measurements applied to fossil plant archives. Global climate mitigation measures currently being debated and trailed include geoengineering solutions involving plant-soil-atmosphere interactions and their capacity to sequester carbon at scale, but their potential and unintended negative consequences are poorly constrained. This talk aims to take the audience on a journey through time, paleoclimates, paleoatmospheres and paleofloras to illustrate how the discipline of paleobotany – the study of fossil plants – has and continues to contribute to vital knowledge on the evolution of Earth's plants, atmospheres and their interactions during past episodes of elevated atmospheric carbon dioxide levels and in turn how such knowledge can inform contemporary policy development for global climate action.

## The role of reactive oxygen, nitrogen and sulfur species as regulators of plant abiotic stress responses

Vasileios Fotopoulos
Department of Agricultural Sciences, Biotechnology and Food Science; Cyprus University of Technology Limassol, Cyprus

Reactive oxygen, nitrogen and sulfur species (RONSS) constitute fundamental components underpinning the dynamic nature of cell signaling systems in plants. Despite their importance in many aspects of cell biology, our understanding of RONSS and their regulation remains a field attracting universal interest. There is solid evidence showing that RONSS participate in a molecular cross-talk towards the coordinated regulation of plant defense responses to environmental extremes. This presentation aims to provide an up-to-date description of main research activities carried out at the Cyprus University of Technology, demonstrating the potentially harmful effects of RONSS and the plant antioxidant defense system involved in RONSS detoxification under abiotic stress conditions. Particular focus is given in the exogenous application of RONSS donors as chemical priming agents, including simultaneous donation of more than one molecules as well as the proprietary hybrid donor NOSH-aspirin, in order to ameliorate abiotic stress-induced damage in model and crop plants.

## Wettstein goes Carrara: integrative species delimitation in hybridising plant groups

Christoph Oberprieler
Evolutionary and Systematic Botany Group, Institute of Plant Sciences, University of Regensburg, D-930404 Regensburg, Germany

Owing to the paramount role of the species rank in evolutionary, ecological and nature conservation studies, the delimitation of species is an essential contribution of taxonomy to biodiversity research. A very active field of research has emerged aiming at a more objective delimitation of species based on molecular data. Being at the interface of phylogenetic analysis (macroevolution) and population genetics (microevolution), classifying species-level biological diversity has made tremendous progress with the availability of multi-locus sequence and/or fingerprint data and the adoption of multi-species coalescent (MSC) models (1). Despite their growing usage in species-delimitation (SD) studies, all of these different approaches have the disadvantage that (a) a biological species concept (BSC) is considered as default and occasional hybridisation or the formation of hybrid zones between species strongly influence the results gained in these studies (2), and that (b) MSC methods can not statistically distinguish structure associated with population isolation vs. species boundaries. The latter problem can be solved by using an integration of genealogical, morphological, ecological, and geographical data for conceptualising species-rank decisions as outlined by von Wettstein in 1898 in his seminal *Grundzüge der geographisch-morphologischen Methode der Pflanzensystematik*, as illustrated in the so-called 'Wettstein tesseract' (3). This auxiliary tool and conceptual rail may support taxonomists in their efforts to attain their goal of an objective, comprehensible and reproducible classification at and below the species-rank. The former problem – the underlying assumption of a strict biological species concept (BSC) in MSC species-delimitation approaches – remains unresolved yet and is especially notorious in higher plants, where hybridisation among closely and even more distantly related evolutionary entities (species) is frequent. In taxonomical respects, it appears necessary to discriminate between *primary hybrid zones* that are formed in the course of parapatric differentiation processes of populations into ecotypes (i.e. ecological speciation, leading to incipient (semi)species, taxonomically best treated as subspecies; 9) and *secondary hybrid zones* formed by the collision of previously allopatrically differentiated species. Again, the joint analysis of genealogical/genetic, morphological, ecological, and geographical data may help to discriminate between the two scenarios and will support taxonomic decisions on subspecies or species rank. Additionally, it may be also necessary to evaluate the prospects of two hybridising lineages for staying independent evolutionary entities in the future (two species) or not (a single species with presently two subspecies). In our presently ongoing CARRARA project funded by the German Research Foundation (DFG) in the framework of the priority programme SPP 1991 'Taxon-Omics: New approaches for discovering and naming biodiversity', we aim at the fast discovery and delimitation of evolutionary significant units (species) in intensively hybridising plant groups without the necessity of additional field work by implementing high-throughput molecular techniques based on herbarium material in combination with the automated extraction of morphological characters and ecological niche-modelling based on museum material. Our approach will be exemplified in three plant groups of the sunflower family (Compositae, Asteraceae) known for their critical taxonomy caused by extensive hybridisation: the *Senecio nemorensis* syngameon (8 species; Europe), the genus *Rhodanthemum* (15 species; NW Africa), and the genus *Baccharis* in Chile (14 species, 26 hybrid combinations).

1) G. Jones (2017) J. Math. Biol., 74, 447-467.
2) A.D. Leaché et al. (2014b) Syst. Biol., 63, 17-30.
3) C. Oberprieler (2023) Taxon, 72, 1-7.

## Who's driving who? Community ecology of trees and ectomycorrhizas

Douglas L. Godbold [1, 2]
[1]Department of Forest Protection and Wildlife Management, Mendel University in Brno, Zemědělská 3, 613 00 Brno, Czech Republic; [2]Institute of Forest Ecology, Department of Forest- and Soil Sciences, University of Natural Resources and Life Sciences Vienna (BOKU), Vienna, Austria

Mycorrhizal fungi form a ubiquitous symbiosis with plants since the emergence of the first land plants 460 million years ago. The majority of tree species of boreal and temperate areas form ectomycorrhizas, which are important for acquisition of nutrients, particularly nitrogen and phosphorus, from both inorganic and organic sources, but also water. In addition, ectomycorrhizal fungi can link trees belowground via a common mycelia network. However, analogous to plant communities, within a forest many species of ectomycorrhizas occur, and differ in both morphological and physiological traits. A single tree species may form symbiotic associations with 50 or more ectomycorrhizal taxa, many of which may be specific to that species, but with the majority being common to many species of trees. This talk will address how trees and tree diversity affects the composition of the ectomycorrhizal community, and how these communities can be linked to ecological function, and the stability of ecosystems. It will consider the major tree species of European forest, but also tree species of extreme environments. Analysis of ectomycorrhizal communities suggests analog to plant communities, that ectomycorrhizal communities can best be described by a passengers and drivers model of ecological function. The talk will also address the question, who is driving who in community ecology?

# COMMUNICATIONS

## Multiple contact sites investigation for chloroplast homeostasis in plant cells

Federica Brandizzi[1], Luciana Renna[2]
[1]Plant Reseach Laboratories, Plant Biology, Michigan State University, 612 wilson Rd, East Lansing, MI 48824, USA; [2]Department of Agricultural, Food, Environmental and Forestry Sciences and Technologies, Università Degli Studi Di Firenze,Viale delle idee 30 Sesto fiorentino (FI), Italy

The plant endoplasmic reticulum (ER) with its extended structure is able to get in contact with multiple organelles forming heterotypic membranes contact sites (MCSs) through mechanisms that are still largely unexplored. Among these interactions the ones between ER and Chloroplast are the less investigate and almost nothing is known about the ER-chloroplast MCS identity.
Through the use of confocal microscopy, proteomic, *in vitro*, *in vivo* interaction, in this study, we demonstrate that an Arabidopsis ER membrane-associated protein and its partner define a functional complex at the ER-chloroplast membrane contact sites (MCSs). In particular, we discovered, through in vivo and in vitro association assays, that the ER membrane-associated protein interacts with the outer envelope membrane (OEM) of chloroplasts, which is where they bind their partner protein. The identification and phenotyping of Arabidopsis ko single and higher order mutant led us to hypothesize that the identified proteins form a functional complex.
We suggest that ER membrane-associated protein and its partner's complex define plant-specific MCSs that connect ER and chloroplasts in a functional way.

## *ACR3* is a key player in arsenic detoxification in bryophytes

Claudio Varotto[1], Enrico Barbaro[1], Daniela Bertoldi[2], Roberto Larcher[2], Mingai Li[1]
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Bryophytes are the sister group to all tracheophytes and the second most numerous group of land plants. Therefore, elucidating in this plant lineage the molecular bases of traits shared with tracheophytes can provide important insights into trait evolution during the radiation of land plants.
Several bryophyte species display high levels of tolerance to arsenic (As), a highly toxic metalloid, but the molecular mechanisms underlying this trait have not been investigated until now. We recently demonstrated that the pathway of arsenic detoxification by vacuolar sequestration of phytochelatin::As conjugates does not play any significant role in As detoxification in the model bryophyte *Marchantia polymorpha* L. (1).
This finding leaves open the question of whether bryophytes rely on a mechanism of active As extrusion, as suggested by the presence in their genomes of genes homologs to *ACR3*, an arsenite transporter responsible for As hyperaccumulation in the fern *Pteris vittata* L.
We, thus, characterized the expression of the single *ACR3* gene present in *M. polymorpha*. The gene is expressed at relatively low levels in all *M. polymorpha* tissues. It is upregulated upon treatment of *M. polymorpha* gametophytes with As, suggesting that it may be involved in As detoxification. To functionally test this hypothesis, we overexpressed the full length CDS of Mp*ACR3* in *M. polymorpha* under the control of the strong constitutive promoter EF1α. Two transgenic lines with high transgene expression were selected from a total of 14 independent transformation events. Additionally, 2 knockout mutant lines corresponding to different target sites were obtained by CRISPR-Cas9 genome editing.
The overexpression lines consistently displayed strongly increased tolerance to both As(III) and As(V) addition to the growth media, while the knockout lines were hypersensitive to both As species compared to the Cam2 WT genotype based on plant fresh weight. Interestingly, the total As content per unit of dry weight in both overexpression lines did not significantly differ from that of Cam2, while both mutant lines accumulated much more As per unit of dry weight than the WT control.
Taken together, these results demonstrate that in bryophytes the *ACR3* gene plays a pivotal role in As detoxification and further suggest that the transporter may be localized to the plasma membrane where it extrudes As from *M. polymopha* cells. The presence of an efficient tolerance mechanism to As mediated by *ACR3* in bryophytes provides compelling evidence in support of the existence of the trait in the common ancestor of all land plants and that it was successively lost in the angiosperm lineage.

1) M. Li, M. Leso, M. Buti, E. Bellini, D. Bertoldi, A. Saba, R. Larcher, L. Sanità di Toppi, C. Varotto (2022) Journal of Hazardous Materials 440: 129844. https://doi.org/10.1016/j.jhazmat.2022.129844.

## Ovule number and fertility are regulated epigenetically during drought stress conditions

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Climate change and global warming will cause reduced crop yield, threatening food security. In this scenario, since most of our food supply is represented by seeds it is crucial to understand how plants cope with drought stress in the reproductive systems. Seeds derive from ovules upon fertilization; therefore, the number of ovules in the pistil determines the number of seeds in the mature fruit, an important agronomic trait. Epigenetic mechanisms were shown to potentially control the ability to respond to stress conditions. For this reason, we investigated which epigenetic mechanisms could control ovule number during drought stress. RNA-directed DNA methylation players, DRM1 and DRM2 were shown to play a role during ovule development. Our data show that in wild-type plants moderate drought stress causes a substantial reduction in ovule number and pistil length, contrary to the double mutant *drm1drm2* which resulted tolerant. Methylome analyses coupled with transcriptomics that included small-RNA sequencing in *drm1drm2* double mutant highlighted a higher level of mir397a, mir398c, and mir408. This miRNA set which impacts copper homeostasis and ROS pathway might have an important role in the resistance to drought stress conditions during reproductive phases.

**Short term exposition to acute cadmium toxicity induces the loss of root gravitropic stimuli perception through PIN2 polar auxin transport in *Arabidopsis thaliana***

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Cadmium (Cd) is a non-essential heavy metal, considered one of the most relevant pollutants, due to its high toxicity and solubility in water (1). In plants, Cd can be absorbed by roots, acting as inhibitor of their growth and development, affecting them from the subcellular to the ecosystem level (2). Root is the first organ that enters in contact with Cd in soil, and consequently, it's considered the primary target of this heavy metal. To prevent Cd-induced damages, plants developed complex mechanisms to avoid its accumulation in cells. Recently, it has been demonstrated that exposure to different heavy metals induces several changes in root system architecture, and increases the activity of root peroxidases, which could induce lignin biosynthesis (3). In this context, the aim of the present work was to investigate the effects of acute Cd toxicity on *Arabidopsis thaliana* L. primary root, exposed for short periods to high Cd concentrations (100 and 150 μM). The effects were studied in both Col-0 and transgenic GFP-marker lines, through integrated morpho-histological, molecular, pharmacological and metabolomic analyses. The obtained results, highlighting that Cd affecting the root apical meristem (RAM) via altering cell expansion in the transition zone (TZ). Moreover, Cd negatively impacted auxin distribution by interfered with the PINFORMED (PINs) family, particularly PIN2, at both transcriptional and post-translational levels. In addition, our results showed that the exposure to high Cd concentrations induce an increase of ROS and an altered organization of cortical microtubules. The disorganization of microtubule orientation pattern, in turn, affects the vesicular transport of PIN2 protein in root, as observed using the marker line *pPIN2::PIN2-GFP*. Moreover, starch and sucrose metabolism, and consequently the gravitropic response, were found impacted. Concerning lignin biosynthesis, by using histological staining, we observed that Cd induces its accumulation, in the region of primary root structure. Globally, our results demonstrated that short Cd exposition affects cell expansion preferentially, altering auxin distribution and inducing ROS accumulation, which results in an alteration of gravitropic response and microtubules orientation pattern. Taken together, our results contribute to understand the primary target of short-term Cd exposition at high concentration.

1) Pinto et al. (2004) Science of The Total Environment, 326, 239-247.
2) Qadir et al. (2014) Rev. Environ. Contam. Toxicol., 229, 51-88.
3) Hélène Lequeux et al. (2010) Plant Physiology and Biochemistry, 673-682.

## Nutrient transfer and metabolism in orchid mycorrhiza, as revealed by gene expression and stable isotope labelling in the *Serapias vomeracea-Tulasnella calospora* association

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As most terrestrial plants, orchids interact with specific soil fungi to form a symbiotic association named mycorrhiza. As compared with other mycorrhizal types, where the fungus provides the plant with mineral nutrients in exchange of photosynthesis derived carbon, orchid mycorrhiza is unusual because the mycorrhizal fungus provides the host plant with organic carbon. This carbon is required for germination of the minute orchid seeds and development of a unique postembryonic structure called protocorm. Carbon transfer in orchid mycorrhiza has been mainly studied in mycorrhizal protocorms, where intracellular fungal coils, or pelotons, are considered the site of nutrients transfer. In addition to carbon, other essential nutrients are needed for plant growth, but limited information on actual transfer to the host plant is available (1-3). We used ultra-high spatial resolution secondary ion mass spectrometry (SIMS) as well as targeted gene expression studies and laser microdissection to decipher metabolism and transfer of nitrogen and sulfur to the orchid protocorm in the model system formed by the Mediterranean orchid *Serapias vomeracea* and the mycorrhizal fungus *Tulasnella calospora*. We revealed that the fungal partner is actively involved in nitrogen and sulfur supply to the host plant, and expression of plant and fungal genes involved in nitrogen and sulfur uptake and metabolism, both in the symbiotic and asymbiotic partners, suggest that transfer most likely occurs as organic forms. These findings pose intriguing questions on the strategies that orchids evolved to obtain reduced forms of essential nutrients from the fungus, at least during the early developmental stages.

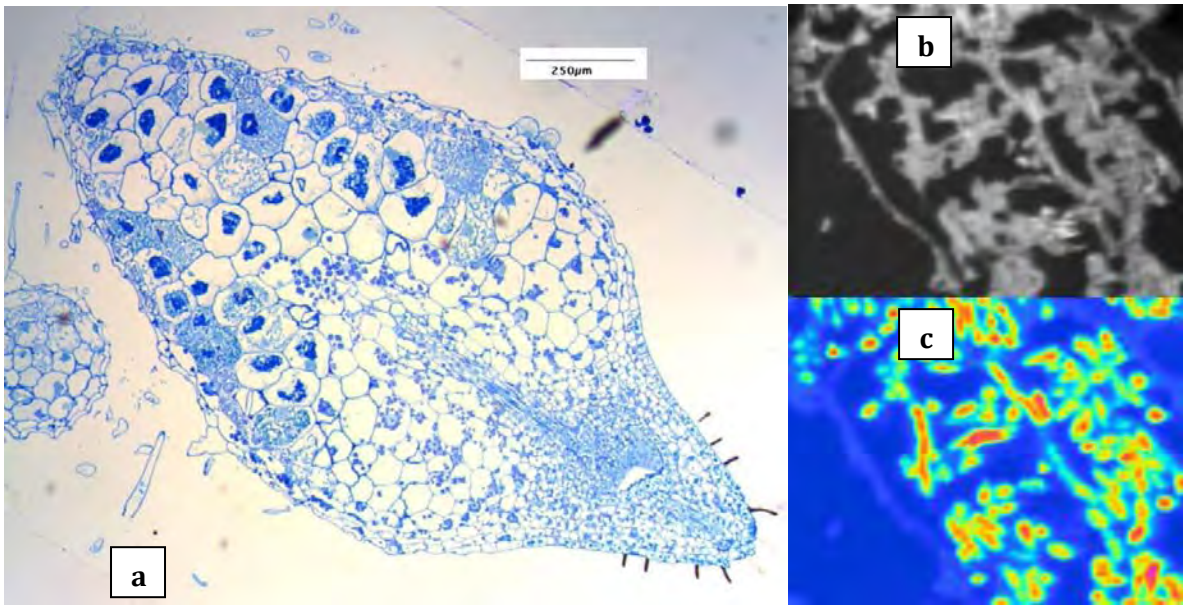


Fig. 1a. Longitudinal section of an orchid protocorm; 1b. a protocorm mycorrhizal cell showing the intracellular mycorrhizal fungal coil; 1c. the same protocorm cell showing $^{15}N$ enrichment, as revealed by SIMS.

1) D.D. Cameron, J.R. Leake, D.J. Read (2006) New Phytologist, 171, 405-416.
2) V. Fochi, W. Chitarra, A. Kohler, S. Voyron, V.R. Singan, E.A. Lindquist, K.W. Barry, M. Girlanda, I.V. Grigoriev, F. Martin, R. Balestrini, S. Perotto (2017a) New Phytologist, 213, 365-379.
3) Y. Kuga, N. Sakamoto, H. Yurimoto (2014) New Phytologist, 202, 594-605.

## From *Marchantia polymorpha* to *Arabidopsis thaliana*: old and new roles of the "multi-tasking" enzyme phytochelatin synthase in land plants

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This contribution will deal, in an evolutionary perspective, with both "traditional" and new roles of the phytochelatin synthase enzyme (PCS), the functions of which still appear shrouded in a degree of mystery. I will illustrate and discuss the five main key-points that characterize this peculiar "multi-tasking" enzyme in plants:

1. PCS is a "papain-like" Clan CA cysteine peptidase, probably evolved from a single ancestral peptidase. In particular, the eukaryotic PCS is a γ-glutamylcysteine-dipeptidyl-(trans)peptidase, and possesses: i) a highly conserved N-terminal catalytic domain, with a "catalytic triad" made up of cysteine, histidine and aspartate placed in specific positions; and ii) a variable C-terminal domain that appears to be involved in thermal stabilization and in modulating the enzyme activity.
2. PCS is constitutively expressed in plants, freshwater/marine algae, lichen chlorophyte photobionts, as well as in other eukaryotes, such as Fungi, some Animalia and Amoebozoa, SAR (especially Bacillariophyceae and Phaeophyta) and, more rarely, Excavata. Humans do not possess PCS. Prokaryotic PCSs have significant sequence homologies with those of plants, but usually lack the C-terminal domain; these "half-size" PCSs have been identified in cyanobacteria and in some β– and γ–proteobacteria.
3. Through its transpeptidasic products – i.e., the metal(loid)-binding oligopeptides named phytochelatins (PCn) – PCS detoxifies toxic heavy metals and metalloids (e.g., cadmium, lead, mercury, arsenic, etc.), but can also help regulate the homeostatic needs of metal micronutrients such as iron, zinc and copper. PCn were isolated in plants for the first time by a German research group (1), although as long ago as 1973 their presence was being hypothesized, extraordinarily for that time, in mercury-exposed tobacco plant leaves by an Italian research group at Pisa University (2). Assuming that, from the Paleozoic era onwards, ancestral bryophytes and tracheophytes spread through paleoenvironments rich in PCn-inducing metal(loid)s, the expression of constitutive and functional PCS might represent still nowadays an evolutionary "memory" of the presence of such metal(loid)s in in such archaic environments. It may be due to these "benefits" in evolutionary fitness that PCS and PCn have been conserved in the vast majority of plants, up to the present day. In this regard, the liverwort *Marchantia polymorpha* represents an interesting model for evolutionary investigations.
4. Being a peptidase (see point 1), PCS can also hydrolyze GSH and GS-conjugated xenobiotics in the cytosolic environment through the cleavage of glycine from GSH. Interestingly, this conjugation not only appears to be quite important for plant detoxification of metal-thiolate complexes or xenobiotics (e.g., halogenated herbicides, monochlorobimane, etc.), but also for the transportation, synthesis, and catabolism of various endogenous substances, such as cinnamic, caftaric and *p*-coumaric acids, anthocyanins, auxins, glucosinolates, etc. The GS-conjugation capacity of plant PCSs is a feature also detected in prokaryotic PCSs, and the breakdown of GS-conjugates may accordingly be considered a primigenial function of PCS, older than the biosynthesis of PCn.
5. A yet limited number of authoritative experimental works (3) is increasingly highlighting the importance of PCS in plant defence against pathogens, such as *Blumeria graminis*, *Phytophthora infestans*, and *Pseudomonas syringae*, essentially in terms of "Microbe-Triggered Immunity" (MTI). For example, *At*PCS plays a part in the production of antimicrobials formed from indole glucosinolates and appears to be essential for callose deposition induced by exposure to the bacterial flagellin (3). At the same time, the lack of *At*PCS alters the phenylpropanoid pathway with variations in the degree of lignification, flavonol content and auxin levels. Interestingly, the *At*PCS's involvement in defending against pathogens, especially in terms of nonhost resistance, appears to have close ties with the above-mentioned PCS cytosolic detoxification of GS-conjugates (see point 4).

In conclusion, PCS is receiving ever-increasing attention in plant biology, not only due to its role in metal(loid) detoxification, but also to its involvement in several other important processes. Advances in this area of knowledge may well help clarify the evolutionary history and function(s) of this ubiquitous "multi-tasking" enzyme for plant organisms.

1) E. Grill, et al. (1985) Science, 230, 674-676.
2) P. Pelosi, C. Galoppini (1973) Atti Soc. Tosc. Sci. Nat. Mem. Serie A, 80, 215-220.
3) N.C. Clay, et al. (2009) Science, 323, 95-101.

## Diversity of marine fungi associated with the ascidian *Halocynthia papillosa*

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Marine habitats cover more than 70% of the planet's surface and host a huge biological and chemical diversity. In recent years, researchers have paid close attention to marine mycology, highlighting fungi's importance in several ecosystem services. It is well known that marine fungi represent one of the main components of sea microbial communities. As their terrestrial counterparts, they play an essential role in recycling resources and energy as part of detritus communities and in several other key ecological processes such as phytoplankton composition and aquatic carbon pump (1). Marine fungi have been retrieved worldwide from almost every type of biotic and abiotic substrates. To date, approximately 1800 fungal species have been detected in marine environments. This represents only a small fraction of the marine fungal biodiversity, as evidenced by the increasing number of new marine taxa described in recent literature (2). Studies on biotic communities revealed several marine fungi with a high degree of substratum specialization, particularly in epi and endo-zoic communities. The nature of the interactions between fungi and hosts is mostly unknown, but secondary metabolites and extracellular enzymes are likely to play important roles in the establishment and maintenance of the relationship. It has recently been proposed that fungal bionts may be involved in host defence mechanisms against microorganisms, viruses, or predators (3). Considering the large gaps in our knowledge regarding marine fungi, the investigation of habitats and substrates, which are neglected from a mycological point of view, appears an essential task to understand their ecology and biodiversity.
The aim of this study was to investigate the culturable mycobiota associated with the ascidian *Halocynthia papillosa* (Giglio Island - Mediterranean Sea); to the best of our knowledge, this is the first mycological research considering this substratum. Globally, a total of 121 strains were isolated using different techniques and cultural media. After the rejection of duplicates, 68 morphotypes were obtained. Taxonomical identification was carried out by a polyphasic approach considering morphological, physiological, and molecular features. Forty-three fungal species and one enigmatic marine protist, *Corallochytrium limacisporum*, were identified among the 68 morphotypes. *C. limacisporum* represents an important species in unravelling the diversification of animals from fungi into the supergroup Opisthokont. The identified fungal species are included in Ascomycota (88.4%) and Basidiomycota (11.6%). Among Ascomycota, the most represented classes were Dothideomycetes (39.5%), Sordariomycetes (28.9%), and Eurotiomycetes (26%). The five Basidiomycota species belonged to Ustilaginomycetes, Agaricomycetes, Tremellomycetes, and Agaricostilbomycetes. Phylogenetic analysis revealed the presence of several new genera and species (8 genera and 10 species) belonging to the orders Lulworthiales (25%), Pleosporales (6%), Venturiales (2.9%), Onygenales (2.9%) and to the phylum Basidiomycota (2.9%). These data confirm that the marine environment is a huge reservoir of unknown fungal species. This work describes for the first time the *H. papillosa* mycobiota, improving our knowledge of marine fungi in the Mediterranean Sea. Nevertheless, further studies are necessary to investigate the biotic relationship between fungi and marine animals. The culture collection of new species and new marine strains represents a valuable potential resource for further biotechnological investigations.

1) A. Amend, G. Burgaud, M. Cunliffe, V.P. Edgcomb, C.L. Ettinger, M.H. Gutiérrez, J. Heitman, E.F.Y, Hom, G. Ianiri, A.C. Jones, M. Kagami, K.T. Picard, C.A. Quandt, S. Raghukumar, M. Riquelme, J. Stajich, J. Vargas- Muñiz, A.K. Walker, O. Yarden, A.S. Gladfelter (2019) MBio., 10, e01189-18.
2) A. Poli, E. Bovio, L. Ranieri, G.C. Varese, V. Prigione (2020) Diversity, 12, 144.
3) O. Yarden (2014) Front. Microbiol., 5, 228.

## What is the role of pollinator-mediated selection on the floral coloration polymorphism of the Neotropical orchid *Epidendrum fulgens*?

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Flower color is one of the most variable traits in nature, and understanding the mechanisms that create and maintain this variation is critical to understanding plant biodiversity. The family Orchidaceae is known for its great floral morphological variation, and also for the high rate of species with deceptive pollination strategies, i.e. plants that deceive their pollinators and do not offer any kind of floral reward. Many studies have associated these strategies with the emergence and maintenance of variation in floral traits at an intraspecific level, since floral polymorphism can reduce the probability of pollinators learning, and thereby reduce the avoidance of rewardless flowers. All the tests for this hypothesis so far failed in identifying an advantage for polymorphic populations. *Epidendrum fulgens* Brongn. (Orchidaceae) is a Neotropical deceptive species whose flowers are visited and pollinated by butterflies, despite not offering any food reward. This species has an evident intrapopulational variation in flower color of inner and outer tepals varying from yellow to orange and red (Fig. 1). Due to these characteristics, *E. fulgens* is an excellent model for studying intrapopulation variation in floral color and the potential mechanisms that maintain this polymorphism. This study aims to understand the role of flower color variation in *E. fulgens*. We tested the hypothesis that intraspecific flower color polymorphism increases the reproductive success of the plants due to a decrease in pollinators' avoidance learning. For this purpose, three clusters of individuals with homogeneous floral color (yellow, orange and red) and one of individuals with heterogeneous floral color were organized and exposed to floral visitors in a common garden in Campinas, Brazil. We performed 12 rounds of cluster exposure, in which the rate of pollinia removal and fruiting were accounted as proxies for male and female reproductive success, respectively. We performed chi-square adherence tests between all values of pollinia removal and fruiting between clusters and between colors in the heterogeneous cluster. Significantly higher pollinia removal rates were observed for clusters with mixed flower color and only yellow flowers (Fig. 2A), which suggests that pollinators can differentiate the flower color variation observed in *E. fulgens*. There was greater visitation of yellow morphs than the others, both in homogeneous clusters and within heterogeneous clusters (Fig. 2), which is a pattern of preference for the yellow color spectrum that is common to many Neotropical butterflies (2). We found no statistical difference in male reproductive success between the yellow and heterogeneous clusters (Fig. 2A), indicating that the presence of yellow flowers in the heterogeneous cluster increased its reproductive success as a whole, indeed the female reproductive success of the heterogeneous cluster was even greater than that of the yellow cluster, although not significant (Fig. 2B). These results suggest that the co-occurrence of plants with different flower colors actually increases the levels of pollen export but not of female reproductive success. Taken together, these data point to a lower pollination efficiency (i.e. a lower proportion of exported pollen reaching conspecific stigmas) in yellow and polymorphic clusters likely linked to an increase in pollinator avoidance learning. Although our results cannot be interpreted as a support for the hypothesis, these represent the first evidence of a change in pollination performances in polymorphic and monomorphic orchid populations suggesting that intraspecific flower color polymorphisms may be under pollinator-mediated selection.


Fig. 1. Inflorescences of different morphotypes: yellow (A), orange (B) and red (C) sepals and petals.

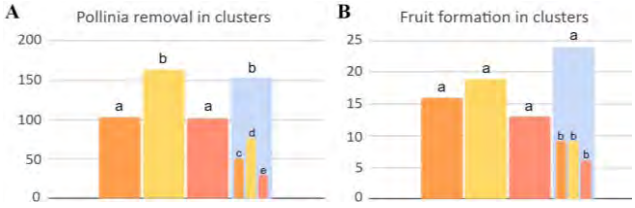


Fig. 2. Bar graphs of male (A) and female (B) reproductive success in the clusters (respectively: orange, yellow, red and heterogeneous).

1) Y. Sapir, M.K. Gallagher, E. Senden (2021).
2) M.R. Weiss, D.R. Papaj (2003) Animal Behaviour, 65(3), 425-434.

## Molecular evidence and environmental niche evolution at the origin of the disjunct distribution in three mountain endemic *Tephroseris* (Asteraceae) of the Mediterranean basin

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Studies on the origin and evolutionary history of closely related plants help to understand patterns of diversity of the mountain flora in addition to providing the basis for their identification. The genus *Tephroseris* includes three micro-endemic taxa with a disjoint distribution in the high mountains of the Iberian Peninsula and on the Maritime Alps. *Tephroseris balbisiana* is native to the Southwestern Alps, *T. elodes* (DC.) Holub to Sierra Nevada, and *T. coincyi* (Rouy) Holub to Sierra de Gredos. These taxa have been treated under different combinations of species or subspecies due to limited morphological differentiation, but comprehensive studies have not been published so far. By combining information from phylogeny, molecular dating and genome size, we demonstrated the taxonomic distinctiveness between *T. balbisiana* and the two Iberian taxa. Although the lack of variability in chloroplast DNA hampered to precise estimation of the diversification events, some of the recovered patterns suggested a recent divergence of *T. balbisiana*, *T. elodes* and *T. coincyi* dating back to the Pleistocene (0.5–2.8 Mya). However, niche modeling supported a geographical overlap between the three taxa during the Last Glacial Maximum (LGM). Moreover, the fragmentation of their larger ancient distribution range, particularly in the lower elevations of the Iberian Peninsula, and migration to glacial refuges in the southwestern Alps, provide the most plausible explanations for the current disjoint distribution within the Mediterranean mountains. Furthermore, based on the evidence we gathered, we inferred that the alpine *T. balbisiana*, as well as the Iberian taxa, should be considered as three distinct subspecies.

## Experimental crosses suggest multifaceted patterns of reproductive barriers among genetically distinct lineages of the *Dianthus virgineus* complex (Caryophyllaceae)

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By virtue of its complex evolutionary history and relevant taxonomic diversity, the genus *Dianthus* has a striking systematic complexity. Various drivers, such as geographic isolation, weak reproductive barriers, and ecophenotypy (i.e., environment-driven morphological differentiation) can greatly contribute in shaping intricate patterns of species' phenotypic variation. In this context, *D. virgineus* L. provides a remarkable example. This species complex was recently revealed to include three geographically structured genetic lineages recircumscribed as *D. virgineus* L., *D. inodorus* (L.) Gaertn., and *D. sylvestris* Wulfen, respectively occurring in the Apennines, Alps, and Balkans (1-3). Despite the overlap at range margins, these lineages show strong genetic isolation, suggesting that intrinsic reproductive barriers contribute to the divergence process. In the autumn 2019, living plants were collected from wild populations of each species and transplanted at the Botanical Garden of University of Calabria. In the two subsequent flowering seasons, a set of vegetative and floral phenotypic traits was measured, and the flowering dynamics was monitored daily for a total of 1,903 flowers (season 2020: N = 407; season 2021: N = 1496). In parallel, 416 flowers were treated with reciprocal crosses to assess the fitness consequences of all possible combinations of intra- and inter-specific breeding. Fitness estimates included measures of pollination success (i.e., fruit-and seed-set), seed quality (i.e., seed size and viability), survival and fertility of *F1* progeny. Phenology and fitness data were used to perform qualitative and quantitative assessments of reproductive isolation among the study taxa. The experimental work suggested a higher phenotypic distinctiveness of *D. virgineus*, while *D. inodorus* and *D. sylvestris* showed a higher extent of morphological overlap. Strong reciprocal barriers were found between *D. virgineus* and *D. sylvestris.* On the other hand, barriers were asymmetrical between *D. inodorus* and *D. sylvestris*, with stronger isolation of *D. inodorus.* Crosses between *D. inodorus* and *D. virgineus* showed overall weak reproductive barriers. These results provide evidence of genetic incompatibilities already acting at the *F1* generation as strong drivers of reproductive isolation between evolutionary lineages, and point to further analyses to dissect additional contributors to this process.

1) H. Luqman, D. Wegmann, S. Fior, A. Widmer (2023) Nature Comm., 14: 1080.
2) G. Domina, G. Astuti, G. Barone, I. Rešetnik, A. Terlević, M. Thiébaut, L. Peruzzi (2021) PhytoKeys, 187: 1-14.
3) G. Domina, G. Astuti, G. Barone, D. Gargano, L. Minuto, L. Varaldo, L. Peruzzi (2021) Taxon, 70: 1096-1100.

## A morphometric study of Calabrian-Sicilian Endemic *Armeria* taxa (Plumbaginaceae)

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The genus *Armeria* (Plumbaginaceae) poses several challenges in its taxonomy and systematics. *Armeria* specimens pose problems of identification due to the intraspecific variability that is observed in this genus. Moreover, the seemingly simple appearance of *Armeria*, characterized by linear-lanceolate leaves in basal rosettes and a naked flowering stem with a capitulum, can be misleading, and these plants lack relevant reproductive barriers among species. The current taxonomy of this genus relies heavily on qualitative morphological data (1). Among the 95 species recorded globally, Italy harbors 17 species, 12 of which are Italian endemics, thus establishing this country as a biodiversity hotspot for this genus. Specifically, Sicily and Calabria are home to four regional endemics (two each) whereas *A. gracilis*, reaches its southern limit on the Pollino massif in Calabria. *Armeria gussonei* Boiss. and *A. nebrodensis* (Guss.) Boiss. are endemic to Rocca Busambra (Palermo) and the Madonie mountains in Sicily, respectively. *Armeria brutia* Brullo, Gangale & Uzunov and *A. aspromontana* Brullo, Scelsi & Spamp. are endemic to Sila and Aspromonte in Calabria, respectively. To resolve the taxonomy of these species and to explore their variability and morphological boundaries, a morphometric study was conducted on 5 populations (*A. aspromontana*, Aspromonte; *A. brutia,* Sila; *A. gracilis*, Pollino; *A. nebrodensis*, Monte S. Salvatore; *A. gussonei*, Rocca Busambra) using 54 morphological characters. All the analyses were performed in RStudio 4.0.3. After data exploration and cleaning, the dataset included 103 individuals × 31 characters. Both the Kaiser-Meyer-Olkin (KMO) test that measure the sampling adequacy (MSA = 0.79) and Bartlett's Sphericity tests ($p < 0.01$) were successfully applied on the dataset, indicating sufficient suitability for factor analysis. For dimensionality reduction, Principal Coordinate Analysis (PCoA) based on the Gower dissimilarity. We modelled the taxa using a Finite Gaussian Mixture Model from mclust package. To do so, firstly, we generated synthetic data from the original data using the synthpop package with a final permuted pMSE between synthetized and orginal data of 0.081, allowing us to use such data in a new matrix of 412 × 31. Secondly, we applied Discriminant analysis based on Gaussian finite mixture given the taxa as prior information to the clustering solution and specifying 5 mixture components. Out of the 14 parametrizations of the covariance matrix, EEI (Equal shape, volume orientation) was found to be the one with the lowest value of BIC for our data. The brier score for the current taxonomic hypothesis was 0.0587 and the log-likelihood was 958.1457. The highest misclassification in the clustering solution was found for *A. gracilis*, *A. brutia*, and *A. aspromontana* whereas only 1 individual was confused between *A. gussonei* and *A. nebrodensis* and no Sicilian individual was confused with the Calabrian populations. Then, we tested the hypothesis of considering as a single morphospecies *A. gracilis*, *A. brutia*, and *A. aspromontana.* We set the number of mixture components to 3. We obtained a brier score of 0.0065 and a loglikelihood of 103.349. The Bayes Factor obtained from the comparison of the two models (BF >> 10) largely indicates that there is more evidence in supporting such new circumscription of the taxa. Lastly we visualized the clustering results using MclustDR function to reduce the dimensionality of the data to two dimensions and we plotted the decision boundaries and density estimates of the species, confirming what has been found numerically for both the solutions. In conclusion, there is a weak morphological support for considering *A. brutia*, *A. gracilis*, and *A. aspromontana* as distinct taxa. On the contrary, there is high support for the independence of *A. nebrodensis* and *A. gussonei*. However, an integrative approach is needed to properly evaluate the taxonomic status of these taxa. Overall, employing statistically robust methods is crucial in understanding the intricate taxonomy of plant groups marked by high variability, seemingly simple appearance, and reticulate evolution.

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1) P.V. Arrigoni (2015) Flora Medit., 25(SI), 7-32.
2) J.F. Aguilar, J.A. Rosselló, G. Nieto Feliner (1999) Syst. Biol., 48(4), 735-754.

## A revision of *Oncostema* (Asparagaceae, Scilloideae) in Italy

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The taxonomy of *Scilla* L. s. l. (Asparagaceae, Scilloideae) is still controversial. The morphological, karyological and molecular differences observed in this group have led some authors (1) to consider *Scilla* as a single genus divided into sections, others (2) to split it into several genera.
*Oncostema* Raf. is a genus that was separated from *Scilla* L. s. s. by its large bulbs, broad, flat leaves, solitary scape, inflorescence with long and broad bracts, prophylls minute, concealed by the bracts, long and straight pedicels, tepals persistent after anthesis, stamen filaments blue, thickened in lower 1/3 and connate at the base, ovary locules 4-6 ovulate, nectariferous ducts covered with short hairs.
These plants live on several types of substrates (carbonate, siliceous, volcanic), in different habitats (cracks in rocks, dry meadows, fertile pastures, river banks, etc.). The distinction between taxa is made difficult by the morphological variability occurring in different growing conditions and by the luxuriance that plants in cultivation could have. Some species have been described on cultivated material of oubtful origin, and in cultivation horticultural hybrids occur. Six species of *Oncostema* are currently known to Italy (3): *Oncostema ceruleum* (Raf.) Speta; *O. dimartinoi* (Brullo & Pavone) F.Conti & Soldano; *O. elongatum* (Parl.) Speta; *O. peruvianum* (L.) Speta; *O. siculum* (Tineo) Speta; *O. ughii* (Tineo ex Guss.) Speta.
Starting from the study of the original material of the names and from literature and field analyses, it was possible to clarify the taxonomic delimitation of the taxa and their regional distribution.
*Oncostema ceruleum* is distinguished by its small size, lax inflorescence, lilac, blue-violet tepals and is endemic to the inland areas of western Sicily; *O. dimartinoi* is small in size, has a dense inflorescence with sky blue tepals and is endemic to the island of Lampedusa (Sicily); *O. elongatum* has a long ciliate leaf margin and bluish-green or yellowish tepals, it is a central Mediterranean species doubtfully native to Italy, occurring in Liguria, Sardegna and Calabria; *O. peruvianum* has linear leaves with an entire or papillose margin, and blue-violet tepals, it is a western Mediterranean species naturalized in Lombardia, Liguria, Tuscany, Campania, Apulia, and Sicily; *O. siculum* has a ciliate leaf margin and sky blue tepals, it is currently known from Sicily, Basilicata, Calabria, and Malta; *O. ughii* is characterized by very broad leaves with an entire membranous margin and is endemic to the island of Marettimo (Sicily).
These ongoing studies will be complemented by additional research focusing on the whole range of the group, with special regard to North Africa, where a large variability has been observed mainly along the central and northern Tunisian coastal regions.

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1) R.M. Almeida da Silva, A. L. Crespi (2013) Flora Iberica, 20, 145-156.
2) F. Speta (1998) Phyton, 38(1), 1-141.
3) F. Bartolucci et al. (2018) Plant Biosyst. 152(2), 179-303.

## Archaeobotany to explore long-term environmental changes and human impact in Italy

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Archaeobotany is the study of plants from archaeological sites. As such, it needs an integrated botanical and archaeological knowledge, with important elements of ecology to understand the relationships between plants and humans. This science studies both microscopical and macroscopical plant remains that testify, from one hand, main traits of the natural environments, and from another hand, selection, uses and economy of past cultures. In such research, the palynological approach is especially useful for detailing the complexity of anthropogenically-driven landscape transformations by discriminating past land uses that, intertwined with climate change, play an essential role in the development of ecosystems over millennial time scales. Furthermore, studying plant macroremains (seeds/fruits, woods, charcoals…) produces direct evidence of foodways, agricultural and cult practices.
Several interdisciplinary studies have been carried out in sites from Neolithic to Roman and Medieval ages located in different regions of Italy. The huge number of studies of plant records from archaeological contexts are collected in the BRAIN database - https://brainplants.successoterra.net/ (1). Among the examples of the multiproxy and integrated archaeobotanical approach centred on archaeological sites is the palynological research at Pantanello (7$^{th}$ century BC – 1$^{st}$ century AD) aimed at reconstructing the environmental and land-use changes in the Metaponto area (S Italy). Pollen data suggest that, even if strictly intertwined, human impact has locally prevailed over climate influence on environmental changes and processes (changes in land use and land cover) and highlighted the importance of anthropogenic overprint on natural processes (2). Another example of archaeobotanical study is the palaeoenvironmental reconstruction of the Stromboli Island (Aeolian archipelago), pointing to the availability of resources in a limited space; moreover, pollen data indicate that the current Mediterranean landscape of Stromboli seems to have originated during the Medieval period (3). Other archaeobotanical case studies will be presented.
Overall, the archaeobotanical research provide us with better knowledge on the past cultural landscapes and the role of human pressure in shaping the current environmental setting and biodiversity.

1) Mariotti Lippi, M., Florenzano, A., Rinaldi, R., et al. (2018) Flora Mediterranea 28, 365-376. https://doi.org/10.7320/FlMedit28.365
2) Florenzano, A., Zerboni, A., Carter, J.C., et al. (2022) Quaternary International 635, 105-124. https://doi.org/10.1016/j.quaint.2022.01.004
3) Mercuri, A.M., Cannavò, V., Clò, E., et al. (2020) Journal of Archaeological Science: Reports 30, 102235. https://doi.org/10.1016/j.jasrep.2020.102235

## Floritaly, an R package to access the Checklists of the vascular flora of Italy

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The Checklists of native and alien Italian vascular flora (1, 2) are considered the gold standard in plant diversity-related research in Italy, having received 971 combined citations in Scopus-indexed journals since their publication. The Checklists provide comprehensive plant names, synonyms, and associated data which are publicly available in digital format through the Portal to the Flora of Italy (https://dryades.units.it/floritaly). The Portal offers additional benefits, including regular updates published semi-annually, links to other digital resources, and a powerful name match tool that aligns any list of names with the Checklists.

Currently, the Checklists data are not included in the growing inventory of packages developed for R and its companion GUI, RStudio, which is an open-source statistical software increasingly used in biodiversity-related research. A Google Scholar advanced search with the query string "Rstudio biodiversity analysis OR analyses" revealed that the number of scientific papers using RStudio for biodiversity analyses more than tripled from 2020 (38) to 2022 (129). Several R packages are indeed available that retrieve taxonomic information from repositories of scientific names and standardise name lists, such as *taxize* and its extension *taxizee*, *taxonlookup*, *rbg*, *taxonstand*, *rotl*. Given the versatility afforded by R in a wide range of analyses and applications, we expect plant diversity researches to benefit from *floritaly*, an R package providing easy access to the Checklists data stored in the Portal to the Flora of Italy. Floritaly can be installed in RStudio by typing devtools::install_github("gibedini/floritaly") in RStudio console. Any use of the package and the data it contains is subjected to a CC-BY license, which requires proper citation of the package and the Checklists (1,2).

*Floritaly* offers two key components: the data itself, and functions for aligning plant names in any given list with the accepted Checklists names along with their associated data.

The data is available in three tables ("dataframes" in R jargon): the master table includes the accepted names and their distribution status in the main administrative subdivisions ("regioni"), while two ancillary tables contain synonyms, accepted names, and fully parsed names.

The package incudes two functions, nameStand() and nameLink(), which enable users to interact with the data tables mentioned above without needing to directly access them. The nameStand() function takes an unrevised list of scientific names, identifies the closest matching names from the Checklists, retrieves the associated accepted name, and returns a standardized name table with four columns: unrevised names, matching names, accepted names, and name distances (computed as Levenshtein distance). The nameLink() function complements nameStand() by associating each standardized name with the corresponding Checklists distribution data through an inner join on the accepted name. By sequentially applying nameStand() and nameLink(), users can effortlessly generate standardized datasets that include accepted names and distribution statuses, starting from unrevised lists.

Additionally, the resulting dataset produced by nameStand() and nameLink() can be further processed and joined with georeferenced occurrence records from public databases such as GBIF (www.gbif.org) or Wikiplantbase(bot.biologia.unipi.it/wpb/italy) using R scripts tailored to specific applications.

1) F. Bartolucci et al. (2018) Plant Biosystems, 152(2), 179-303. https://doi.org/10.1080/11263504.2017.1419996
2) G. Galasso et al. (2018) Plant Biosystems, 152(3), 556-592. https://doi.org/10.1080/11263504.2018.1441197

## Seed germination responses of annual back dune species to current and future climatic scenarios

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Plant species living in coastal sand dunes are subject to environmental conditions limiting their survival, including high soil temperature and variation in water availability. Climate change could further exacerbate these environmental conditions by changing rainfall patterns, especially reducing water availability in the autumn-winter period and an increase of temperatures during the whole year. Moreover, climate change may disrupt the natural seed dormancy cycles, determining an advanced or delayed seedling emergences under unfavorable establishment periods. Annual plant species may be particularly affected by environmental conditions and their variation due to climate change, as they must regenerate each year by seeds.
Here, we studied seed responses of annual dune species to environmental cues simulating current and future climatic conditions in the laboratory. We sampled three annual species typically dominating back dune annual grasslands in the central Mediterranean coasts: *Festuca fasciculata* Forssk. (Poaceae), *Marcus-kochia ramosissima* (Desf.) Al-Shehbaz (Brassicaceae), *Silene canescens* Ten. (Caryophyllaceae). Seeds were collected in late spring from approximately 100 randomly selected individuals for each species and either immediately sown on plain agar to quantify the dormancy level at dispersal (fresh seeds) or after-ripened (~20°C, 50% RH) for 6 and 9 months. Seeds were exposed to 4 different average temperatures (10, 15, 20, 25), 2 temperature regimes (constant vs alternating) and 2 light conditions (diurnal light and constant dark) for a total of 32 experimental treatments.
Fresh seeds reach very low final germination proportions (3-4%) at any condition in all species, while after-ripening overcomes dormancy allowing for wide germination responses at most temperature and light conditions. Nevertheless, *M. ramossima* seeds germinate at low percentage even when after-ripened, thus this species probably exhibits deeper dormancy compared to the other two annuals. Seeds were insensitive to temperature and light regimes, with near equal germination proportions for constant, alternating, full dark or light exposed seeds. Germination temperature optimum was around 20°C, which is slightly higher compared to the majority of Mediterranean species. This elicits a prompt germination of non-dormant seeds in early autumn, allowing seedling emergence immediately after the first rains as demonstrated by our field phenology studies.
To further understand seed responses under stressful conditions, we will explore the effect of different water potential levels (0, -0.5, -1 MPa) and high temperatures (>30°C) which may be experienced by seeds due to climate change.
Studying seed responses to current and future climatic scenarios, will allow to identify the thermal and water potential germination thresholds, as critical information to forecast the germination risk and to define future potential threats to the long-term persistence of back dune species avoiding biodiversity losses.

## UAV monitoring of the spread of *Juniperus macrocarpa* on the dunes of NW Tuscany

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*Juniperus macrocarpa* Sm. is a shrub or small tree typical of stable dunes and cliffs in the Mediterranean region (1). The species is diagnostic, constant, and dominant of EUNIS habitat N1B with a VU (vulnerable) status (2). Although not considered an entity at risk, its distribution and habitat in Tuscany show a great discontinuity, due to high anthropic pressure and/or erosion. Here *J. macrocarpa* in some areas has almost vanished due to erosion, in others, it is threatened by tourist pressure, in others still, instead, is expanding. In this survey, we report data related to the fast spread of juniper observed in Marina di Vecchiano (PI), a sandy coast in the north part of the MSRM Regional Park. In this area, *J. macrocarpa* is spreading from the internal consolidated dune to the foredune. For the investigation, an area of about 100 large and 300 m depth and with relatively stable shoreline dynamics in the last 10 years, was chosen. Since 2010 the beach in front of the mobile dune has been protected. This embryonic belt has been quickly colonized by the typical pioneer species of the shifting dunes but also by juniper. For monitoring, the use of high-resolution orthophotos (20 cm pixels) (years 2016, 2019) and UAV technology with 0.7 cm pixel resolution photos (years 2021, 2022, 2023) were added to the ground control. The surveys were carried out along 5 transects 10 meters wide and deep for the entire length of the dune sector. This was separated into 5 belts: embryonic + mobile dune (A), consolidated grassland (B), consolidated juniper (C), and consolidated mixed wood (D) (Fig.1).

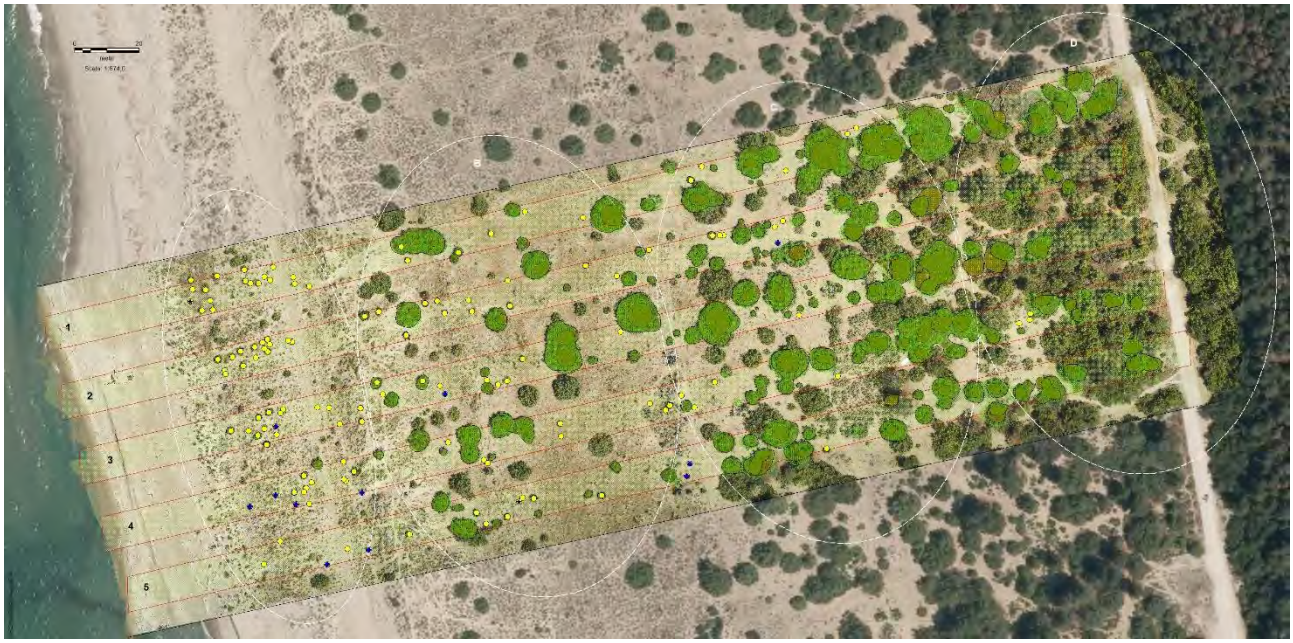


Fig.1. Overlapping of the different orthophotography layers (2016-2023) and identification of juniper (yellow dot = new specimens; green polygons = pre-existing specimens).

UAV has proven to be a useful and powerful tool in remote tracking. The first results show a fast colonization of the juniper in A belt. Before the protection of this stretch of beach, only species typical of shifting dunes could be found. Since 2016, the first juniper seedlings appeared, and, in this belt, the population increased by 400 %. This increase is reduced by passing to the inner belts (B= 120%; C=16.6%; D= 9,1 %). This dynamics of colonization, even if conforming with the pioneer behavior of *J. macrocarpa*, does not seem in line with the initial constraints of the species in thermo-Mediterranean environments (strong summer aridity and strong insolation). The coastal environment of northern Tuscany (Meso-Mediterranean) can probably mitigate the aforementioned limiting factors.

1) E. von Raab-Straube (2014+) Gymnospermae In Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity. – Published at http://www.europlusmed.org [accessed 15/05/23].
2) M. Chytrý et al. (2020) Applied Vegetation Science, DOI: 10.1111/avsc.12519

## Dolichens project: a dynamic inventory of the lichen biota of the Dolomites

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The Dolomites, located in the Eastern Alps and appointed as a UNESCO site, are increasingly impacted by climate change and anthropization. Under this scenario, many species may face severe extinction risks, resulting in the loss of hidden biodiversity that is still partly undiscovered. This is the case of many neglected organisms that play relevant ecological roles contributing to ecosystem functioning, such as lichens. While the lichen biota of the Alps is relatively well known, a specific synthesis for the Dolomites is still missing, despite the long tradition of lichen exploration in this area. This hampers appropriate conservation and the recognition of poorly known taxa. For this reason, we launched the "Dolichens project" aiming at recording all the available lichen data for this area and at filling the gaps with new fieldwork. First, we gathered all the available literature and herbarium specimens since the 19$^{th}$ century, as well as unpublished or grey-literature data to compile a baseline inventory of the lichens of the Dolomites. Second, we started new fieldwork prioritizing protected areas and including historical localities as well as new localities that still miss lichen exploration. Data were stored in a dynamic database (https://italic.units.it/dolichens), which will be continuously updated. Each record was georeferenced and displayed on a web-GIS map. Currently, we have stored 59098 records referable to 1565 taxa, reported from the early 1800s to the present day, from hilly to nival belts, and corresponding to half of the species known for the whole Alpine chain. This study also reveals the occurrence of several poorly known taxa, as in the case of *Aspicilia bricconensis* Hue and *Thelidium paneveggiensis* (Servít) ined., reported only in the type locality and whose distribution and taxonomy should be further investigated. Overall, we expect that this open floristic inventory will contribute to composing and maintaining the puzzle of a lichenological memory of the Dolomites, allowing us to track its lichen biota across ages, facing the challenge of rapid global changes.

## Palynological evidence of climatic variability and reforestation in Italy during the Little Ice Age (LIA)

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The last thousands of years include important climatic fluctuations in the Mediterranean basin. These anomalies impacted on the vegetation and are often associated with change in land use across Europe. They are attested during the so-called Homeric climatic Oscillation (8$^{th}$ – 6$^{th}$ cent. BC), Roman Optimum (2$^{nd}$ cent. BC – 5$^{th}$ cent. AD), Medieval Warm Period (MWP, mid-10$^{th}$ – mid-13$^{th}$ cent. AD), and Little Ice Age (LIA, 15$^{th}$ – 19$^{th}$ cent. AD). In particular, the latter occurred between the Late Medieval and Early Modern period during which Europe experienced cooler temperatures and altered precipitation patterns. During this timeframe, historical sources confirm that Italy faced more frequent flood events of the Tiber River and alluvial phases in the northern Apennines, as well as advance of glaciers and cooling in Europe (Fig. 1).
Such prolonged cool and wet climatic conditions altered the vegetation dynamics and caused shifts in plant distribution and community composition across Italy. The main effect of the LIA was the expansion of cold-tolerant plants into lower elevations and latitudes, and the change of forest ecosystems with the contraction of thermophilous trees to the advantage of conifers. Agricultural practices also faced challenges due to shorter growing seasons and frost events, and farmers had to adapt by adopting cold-tolerant crops and changing planting and harvesting times.
We present palynological data from lakes of Latium and Sicily, showing the effects of LIA climatic change on the vegetation patterns of the investigated regions. The period of increased precipitation caused the rise of lake water level (1, 2, 3) with the increase of either local hygrophilous or arboreal vegetation. The rapid increase in arboreal pollen testifies the general expansion of forests, although vegetation continued to be modeled by human pressure. Population faced the effect of the LIA in addition to the multiple waves of pandemic that devastated all Europe since the 14$^{th}$ cent. AD after the arrival of the Black Death. We suggest that the combination of changing climatic conditions and adaptive strategies of human communities resulted in: a) increased biomass shown by the increase of tree pollen and b) different vegetation response and resilience of plant communities. The phenomenon will be further investigated in Abruzzi and Calabria regions in the frame of the PNRR CN 5- National Biodiversity Future Center – Activity 4.4: Scenarios of Area-based conservation planning and management, that aims to reconstruct changes in both animal and plant variability due to natural and/or anthropogenic events in protected areas.

Fig. 1. La laguna di Venezia ghiacciata. Dipinto di Francesco Battaglioli (1788).

1) S. Mensing, I. Tunno, L. Sagnotti, F. Florindo, P. Noble, C. Archer, S. Zimmerman, F.J. Pavon-Carrasco, G. Cifani, S. Passigli, G. Piovesan (2015) Quaternary Sci. Rev., 116, 72-94.
2) L. Sadori, C. Giraudi, A. Masi, M. Magny, E. Ortu, G. Zanchetta, A. Izdebski (2016) Quaternary Sci. Rev., 136, 173-188.
3) L. Sadori (2018) Quaternary Sci. Rev., 202, 30-44.

**Phylogenetic and trait-based correlates of extinction risk in Italian flowering plants**

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Plant species differ in their biological susceptibility to extinction. However, it is yet unclear which patterns are common across lineages or ecosystems, and which are taxon-specific or restricted to specific context, with consequences to conservation practice. Moreover, whilst extinction risk is not a heritable trait, there is emerging evidence that it is not randomly distributed across the tree of life, likely because extinction-proneness is shared among closely related species.

Here, we investigated the contribution of spatial predictors, adult plant traits, reproductive traits, and habitat type on the extinction risk of Italian flowering plants using phylogenetic generalized linear mixed models accounting for the shared evolutionary history among species. Specifically, we modelled extinction risk as an ordinal index based on Red List categories of the Italian vascular flora compiled by the working group for Nature Conservation of the Italian Botanical Society. We then collected data for 472 angiosperm species on endemic status, range size, and biogeographic regions potentially reflecting population size/number and dispersibility; we also considered life cycle, woodiness, and plant height as proxies of generation time and competitiveness; pollination type, reproductive system and seed mass accounting for sexual reproductive specialization and regeneration strategies were also included; finally, we considered habitat type, elevation range and spatial aggregation of individual plants accounting for habitat breadth and human footprint.

We show that in all models extinction risk was affected by the phylogeny, suggesting that threatened species are non-randomly distributed across the phylogeny. Extinction risk is highest in narrowly distributed species (though not necessarily endemic), with restricted elevation range, especially from the Mediterranean region thriving in coastal, humid, or semi-natural habitats.

Overall, extinction risk in the Italian flora affects all species living in fragile and threatened habitats regardless of their reproductive or vegetative traits but with similar susceptibility to extinction among phylogenetically related species and with restricted ranges and habitat breath. Not surprisingly, extrinsic predictors of extinction risk (habitat type as a proxy of human footprint) indicate that habitat degradation is one main threat to plant species. However, in light of the phylogenetic clustering in extinction risk, we also show that extinction-proneness is shared among closely related species likely because similar species share intrinsic biological features.

We conclude that whilst extrinsic and spatial predictors undoubtedly explain extinction risk, it is still not entirely clear which intrinsic plant traits could explain phylogenetic clustering of susceptibility to extinction. The integration of evolutionary and biological data would thus facilitate more effective conservation and management strategies.

## Alpine Botanic Gardens as treasure chests of biodiversity for the quasi in situ conservation of Plant Genetic Resources

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The alpine flora is threatened by climate change and land-use changes. In response, several seed collection campaigns have taken place in the European Alps in the last two decades to expand *ex situ* collections[1]. Long-term seed conservation involves storage in dry and cold conditions in germplasm banks (15 °C, 15% RH and cryopreservation at -18° C). However, this approach faces several key challenges. For instance, i) the collection of samples in alpine area can be an extremely time-demanding activity, ii) the target populations may have a very short time span for seed collection, produce low-viable seeds or seeds that are short-lived in seed banks, and iii) seedling requirements and propagation protocols are in most cases unknown. Moreover, genetic-based conservation units are still undefined for most species and this could lead to a severe underrepresentation of a species' actual genetic diversity in germplasm banks. A possible way forward in order to overcome these issues is to integrate *ex situ* conservation with living collection facilities. Botanic gardens and arboreta have been identified as a reservoir of Plant Genetic Resources (PGR)[2]. Specifically, alpine botanic gardens have the additional advantage of maintaining living collections acclimated to the local alpine environmental conditions. Most of these facilities maintain hundreds of species in a semi natural habitat with no (or very low) anthropogenic input. This approach is known as *quasi in situ* conservation[3] and aims to preserve neutral and adaptive genetic diversity in multiple *ex situ* living collections. In this study, we conducted a thorough survey of botanic gardens scattered across the European Alps in order to assess the current biodiversity preserved in their living collections. Furthermore, we designed a semi-structured questionnaire to collect technical information on horticultural practices (e.g., propagation, mowing, composting, seed collecting), dissemination initiatives and partnerships with research institutes. Cumulatively, these facilities preserve more than 8000 accessions for ca 2000 plant species belonging to more than 80 plant families. The managing authorities are heterogeneous: two gardens are part of natural history museums, two are managed by a regional authority for agriculture, one by the national ranger service, one by a national park, and six by municipalities. Their horticultural techniques follow common organic farming practices, including manual removal of pests and biological control, composting of green results, mulching or hay cutting, soil aeration. The managing level varies vastly, from a full "plant bed" approach up to semi-wild low-management areas. In general, elevation and microclimate have been reported as the crucial factors for collection maintenance. Local geomorphological feature allows cultivating species occupying different elevational zones in a relatively small area. Seed collection and curation are widely performed in alpine gardens, but mostly for local propagation purposes, although seed exchanges have also been reported. In conclusion, we strongly recommend the integration of germplasm facilities with alpine gardens in order to pair *ex situ* PGR conservation with living collections in a *quasi in situ* environment. Next, we plan to perform a gap analysis of preserved alpine species by cross-checking living specimens with seed lots preserved in germplasm banks. The gap analysis results will enlighten the species barely or totally neglected by germplasm banks and for which alpine botanical gardens could give an immediate contribute in terms of seed lots.

1) J.V. Müller, C. Berg, J. Détraz-Méroz, B. Erschbamer, N. Fort, C. Lambelet-Haueter, E. Breman (2017) Journal of Mountain Science, 14(4), 806–810.
2) T. Abeli, S. Dalrymple, S. Godefroid, A. Mondoni, J.V. Müller, G. Rossi, S. Orsenigo (2019 Conservation Biology, 34(2), 303-313.
3) S. Volis, M. Blecher (2010) Biodiversity and Conservation, 19, 2441-2454.

## Magnetic and chemical biomonitoring with lichens and trees leaves for the conservation of cultural heritage

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Biomonitoring of air pollution applied to cultural heritage is an innovative strategy of preventive conservation from damage caused by air pollutants. The main objective of this study is to evaluate the presence of pollutants, e.g. particulate matter and potentially toxic elements (PTEs), characterizing their distribution and peculiarities using air biomonitoring techniques. Lichens and leaves were used for this purpose: lichens were firstly collected in remote areas and exposed in the sites of interest for three months, the leaves, when available, were collected on site. The biomonitors were characterized by magnetic analysis, providing information on the abundance, composition, and grain-size of their ferromagnetic burden, which is often linked to the presence of PTEs. The main magnetic parameters investigated were the magnetic susceptibility, a sensitive indicator of the magnetic minerals' concentration, and the hysteresis properties that define the characteristics of magnetic particles. Afterward, chemical analysis using multi-element techniques such as ICP-MS was used to determine the content of PTEs. The first application of these techniques, in the light of the work in Villa Farnesina in Rome (1), and out of the usual urban context, was at the Peggy Guggenheim Collection of Venice (Fig. 1), where the main source of PM in urban areas, i.e. vehicle traffic, is missing. Both indoor and outdoor areas were investigated, along a transect from Canal Grande to the inner garden of the museum. The magnetic contribution differed significantly between indoor and outdoor transplants. Moreover, out of a total of ten elements (Fe, Al, Ba, Cr, Zn, Cu, Sb, N, V, S) analyzed, Sb stood out on the outside and Zn on the inside of the museum. The results from the Guggenheim Collection highlighted unusual sources of pollution, likely related to the industrial activities in Venice, i.e. the artistic glass factories. Moreover, it revealed the role of indoor sources in influencing airborne zinc's diffusion, possibly related to mural painting and the use of zinc-containing filters for the conservation of the masterpieces. Another study case is the Palatino Hill of Parco Archeologico del Colosseo, the main archeological site of Rome. Here the buffering effect of trees and shrubs along the areas facing a busy road (Via dei Cerchi) was tested, along with an ongoing biomonitoring study with lichen transplants, that are still exposed outdoors and indoors the Schola Praeconum (Fig. 2). The deposition of magnetic particles on *Quercus Ilex* leaves was particularly high and mainly linked to street dust close to the road, while it was significantly lower on the shrub leaves of the Palatine hill, mostly influenced by geogenic dust. These results highlight the role of trees in the characterization and mitigation of airborne pollution from vehicular traffic. The ongoing work focuses on other study cases: the Museo Nacional de Bellas Artes and the Museo Histórico Nacional, both located in Buenos Aires, Argentina, for comparing the efficacy of two lichen species.

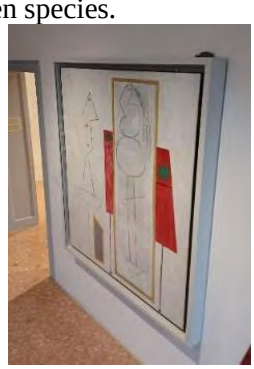

Fig. 1. Lichen transplant on "The Studio" by Picasso.

Fig. 2. Lichen transplant in Schola Praeconum.

1) A. Winkler, T. Contardo, V. Lapenta, A. Sgamellotti, S Loppi (2022) Sci. Total. Envir., DOI: 10.1016/j.scitotenv.2022.153729

## Influence of the structure of resident plant communities and abiotic characteristics on the performance of the invasive alien species *Senecio inaequidens*

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Invasion success by alien plants is often considered to be context dependent. Mainly, it has been described to be associated with specific traits of alien species, environmental conditions of the invaded range or a combination of both. Both biotic and abiotic conditions in the invasive range may collectively have a role in preventing the successful establishment and invasion of alien species. Among some of the hypotheses put forward to explain these effects, the biotic resistance hypothesis posits that a recipient communities would be resistant to invasive species if there are no available resources to be used by alien species, leading to stronger competitive interactions that can limit its performance or establishment in a new range. In other words, the hypothesis predict that the success of an alien species would decrease in high diverse native communities and/or if the alien is functionally/phylogenetically closely related to the resident species due to competition for available or similar resource uptake strategies respectively. However, these patterns tend to be species-specific, and are not always confirmed, leaving open questions on whether the structure of the community and if functional/phylogenetic differences between native and alien species are valid predictors of invasion success (1).
We explore these questions using the South African ragwort (*Senecio inaequidens* DC.) as a model species. *S. inaequidens* is a perennial chamaephyte of the family Asteraceae, native to the Drakensberg region in South Africa and Lesotho, and currently invasive in many countries in Europe and across the world, where it was accidentally introduced by the end of the 19$^{th}$ century. It is mostly found in ruderal and anthropic habitats, such as roadsides, railways and vineyards, but also in more natural and semi-natural contexts, such as arid meadows, riverbanks, rocky slopes, and sand dunes, where it is likely to compete with native resident vegetation for resources.
To evaluate the influence of abiotic conditions and biotic interactions with the recipient community on the performance of the invasive alien species *S. inaequidens*, we identified three different semi-natural habitats where the species is known to grow in Northen Italy and where uninvaded areas also co-occur: riverbanks, arid meadows and restored quarry sites. In each habitat we collected data on main soil chemical-physical characteristics (pH, total N, assimilable P, organic C) and the resident native communities (species composition and cover), using 1×1 m plots. The performance of the target alien species in these habitats was evaluated considering its cover and main functional traits (canopy height, SLA, number of capitula). We then calculated the mean weighted phylogenetic distance (CMWPD) between *S. inaequidens* and the plant species of the resident communities. By means of linear mixed models, we evaluated the influence of the structure of resident native communities (CWMPD and species richness), habitat type, and of soil characteristics on the performance of *S. inaequidens*. Our preliminary results show that, contrary to our expectations, *S. inaequidens* cover and reproductive output were higher in species-richer and more related communities (i.e. when CWMPD between *S. inaequidens* and the community was lower), while its height and SLA were not affected by the structure of resident communities. Soil characteristics did not seem to have a significant influence on the species performance since all habitats were characterized by poor nutrient content and sandy soils. The fact that *S. inaequidens* performed better in more closely related and richer communities might be ascribed to a disturbance effect always present in the sampling sites. Therefore, facilitation more than competition may play a key role in shaping the community structure. Further research into the functional structure of the invaded communities might give additional insight on the drivers that promote *S. inaequidens* performance.

1) E.Z. Daly, O. Chabrerie, F. Massol, B. Facon, M.C.M. Hess, A. Tasiemski, F. Grandjean, M. Chauvat, F. Viard, E. Forey, L. Folcher, E. Buisson, T. Boivin, S. Baltora-Rosset, R. Ulmer, P. Gibert, G. Thiébaut, J.H. Pantel, T. Heger, D.M. Richardson, D. Renault (2023) Oikos, 1–29. https://doi.org/10.1111/oik.09645.

## Impact of the invasive alien macrophyte *Ludwigia hexapetala* on freshwater ecosystems

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Freshwater habitats are highly susceptible to biological invasions; in fact, they are often disturbed by numerous anthropic pressures that generate unstable environmental conditions favoring the spread of alien plant species (1). One such species is the American macrophyte *Ludwigia hexapetala* (Hook. & Arn.) Zardini, H.Y.Gu & P.H.Raven, which was first introduced in France for ornamental purposes, from where it then spread to other European countries, including Italy. This macrophyte invades both aquatic and bank habitats of lakes, rivers, and canals, thanks to its exceptional morphological plasticity. Indeed, *L. hexapetala* shows two different morphotypes: a pioneer, aquatic morphotype with floating rosettes of round leaves, and a later, terrestrial morphotype with elongated flowering stems. Although it is known that *L. hexapetala* releases allelopathic substances into the surrounding environment, hindering seed germination and growth of other plant species (2), only fragmentary information is available on its actual impact in in the areas that it invades.
This study aimed to assess the impact of *L. hexapetala* on environmental parameters and native plant communities occurring in the invaded habitats. A total of 50 surveys were carried out in waterbodies in north-central Italy where *L. hexapetala* was reported or that it could potentially colonize. The study area included Superior and Inferior Lake of Mantova, Bracciano Lake, the Latium stretch of the Tiber River, and canals in Torvaianica and Latina. In each survey, presence and cover (%) of *L. hexapetala* and each macrophyte species occurring, along with some environmental parameters (air and water temperature and luminosity, water dissolved oxygen) were noted. Results showed that the occurrence of aquatic populations of *L. hexapetala* severely alters water quality. In fact, this species can produce extensive floating mats on the water surface that interfere both with the penetration of light into the water column, and with oxygen exchanges at the water-air interface. This, combined with the species ability of effectively capturing most of the oxygen dissolved in water, generates dark and anoxic conditions in water that are unfavorable to the survival of other aquatic plants. Indeed, aquatic plant diversity was significantly reduced by the presence of *L. hexapetala*: as the cover of the alien species increased, there was a significant decrease in Simpson Diversity Index. In particular, the occurrence of native species, such as *Potamogeton nodosus* Poir., *Ceratophyllum demersum* L. and *Myriophyllum spicatum* L., was severely limited by the dominance of *L. hexapetala*, suggesting that aquatic plants, being the first to face the alien species in its colonization phase, are more susceptible to its invasion. Even along the banks, the terrestrial morphotype of *L. hexapetala* can produce dense and tall populations (up to 1 meter in height) that significantly limit the passage of light and heat from the air to the soil surface, potentially interfering with the growth of other plants. However, no significant impact on bank plant diversity was observed, suggesting that bank communities are more resistant to the invasion of this alien species. Indeed, they are generally more structurally compact than the aquatic ones, establishing a kind of “wall" against *L. hexapetala* colonization. In particular, the native *Phragmites australis* (Cav.) Trin. ex Steud. plays an important role in counteracting the invasion of this alien species, since it forms dense populations in the transition area between aquatic and bank habitats, hindering the establishment of *L. hexapetala* propagules arriving from nearby invaded aquatic areas.
Overall, this study showed that, although *L. hexapetala* represents an important threat to native aquatic plant communities, it may encounter resistance along the banks, where communities of native plants may oppose its colonization. This information becomes particularly valuable for all those environmental managers of waterbodies threatened by *L. hexapetala*, where they could favor the vegetation types most capable of resisting the invasion of this alien species to control its spread and preserve these habitats.

1) L. Lazzaro, R. Bolpagni, G. Buffa et al. (2020) Journal of Environmental Management, 274, 111140.
2) S. Dandelot, R. Verlaque, A. Dutartre, A. Cazaubon (2005) Hydrobiologia, 551, 131-136.

## Effects of the invasive alien *Salpichroa origanifolia* on the soil seed bank of a mixed forest (San Rossore, Migliarino, Massaciuccoli Regional Park, Pisa)

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*Salpichroa origanifolia* (Lam.) Baill. is a perennial herb of the Solanaceae family, native to the temperate regions of South America, where it grows from 0 to 2500 m a.s.l., often behaving as a ruderal species. The species is naturalised in Europe, is not listed as an invasive alien species of EU concern and is not included in any of the EPPO lists. Although reports from European countries are scarce, *S. origanifolia* has been found spreading rapidly in southern Switzerland and the Canary Islands. In Italy, the species is present in most regions and its status was recently changed from naturalised to invasive in Campania and Calabria (1). Pathways of introduction can be unintentional, through trade in seeds and potted plants containing seeds or stem fragments, or intentional, as ornamental, or melliferous plant. The first report for Tuscany dates to 1923, but around 2000 the species was still considered rare (5). *Salpichroa origanifolia* was not present in San Rossore before 2000 (2), and it was accidentally found in cut stone pine plantations and near the buildings at the entrance to the reserve in 2008 (3). Ten years later, *S. origanifolia* showed a clear invasive habit, forming dense stands at forest edges, under isolated trees and inside the forest, especially in correspondence of fallen trees.
Phenological and morphological traits of this species contribute to its invasiveness. Shoots vegetate from March to December, and the flowering period is very long, from April to October. Plants spread rapidly producing scrambling and rooting aerial stems, and two types of rhizomes: fine fleshy, 3-5 mm in diameter, just below the soil surface, and around 7-mm-thick deep growing woody rhizomes.
This study assessed the competitiveness of *S. origanifolia* against native forest species, by analysing the weekly emergence and the biomass achieved after 11 weeks. The soil seed bank was collected in the forest of San Rossore, from two sites with different levels of *S. origanifolia* invasion: one with a low (LD So) and one with a high (HD So) density. Two additional treatments, arranged in a split-plot design, were imposed: light conditions (full sunlight/canopy shade) and the presence or absence of *S. origanifolia* rhizomes. The light treatment was set to test if the invasiveness of *S. origanifolia* and its effect on the soil seed bank varied between undergrowth and open areas. Results showed that, in both sites, the presence of *S. origanifolia* rhizomes did not inhibit either the germination rate or the biodiversity of native plants. However, in the full light treatment, the presence of *S. origanifolia* increased the germination rate, suggesting that shading by this invasive species had an initial positive effect on the understorey species. Results concerning biomass differed between sites. In the low-density site, we observed a difference in the biomass of native species between treatments with or without *S. origanifolia*. In the former case, the biomass was reduced by about a half. In the high-density site, the biomass of the native species was generally very low, independently on the presence of *S. origanifolia* rhizomes. This is to impute to the emergence of *S. origanifolia* seedlings from the soil seed bank, which were very competitive against native species. In fact, their biomass was fivefold higher than that of the native seedlings. Furthermore, the biomass achieved by a single *S. origanifolia* seedling was 2 g, whereas that of a seed bank seedling was just 0.05 g (Fig. 1).

Fig. 1. Seedlings of *S. origanifolia* and native species.

1) V.L.A. Laface, C.M. Musarella, A. Cano Ortiz, R.Q. Canas, S. Cannavò, G. Spampinato (2020) Plants, 9, 1181
2) F. Garbari (2000) Atti della Società Toscana di Scienze Naturali, Memorie – Serie B, 107:11-42.
3) C. Orlandi, I. Arduini (2010) Informatore Botanico Italiano, 42(2), 473-477.

## Forest monitoring updates: physiological indicators of tree health for detecting and understanding a changing environment

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Crown defoliation is the main parameter used to assess forest tree health. It has been adopted as indicator of tree vitality since the beginning of forest monitoring in Europe, in the '80s of the past century. In Italy defoliation assessment, carried out since 1996 in the national monitoring forest plot network, has made available data series on the health of the national forests, providing a robust representation over time and space. Recently, defoliation was found to be a sensitive indicator of impacts of climatic anomalies, such as recurrent harsh droughts, summer heat waves, wind storm and other abiotic disturbances, such us air pollution and atmospheric depositions, as well as biotic attacks (i.e., bark beetle in *Picea abies* (L.) H.Karst. stands), concomitant or consequential to severe climatic events. Events of this type have become frequent, intense and geographically widespread. The national extensive forest monitoring network, with 260 permanent plots (*c.* 5000 trees) distributed in all Italian regions, representative of the main forest types, allowed to know the extent and intensity of the damage (i.e., defoliation) by climatic events and other environmental factors, and the subsequent recovery processes put in place by some trees (and species) after the damage (i.e., reduction of the defoliation). In view of the fast climate changes, with increasing frequency of extreme events, and with short time for physiological recovery of trees, the need to consider the physiology of trees in forest health assessment, so in monitoring programmes, has emerged. The effects of defoliation on plant physiology play a central role in the tree-environment interactions and in plant species responses to climate changes. New indicators of tree health based on plant physiology, as photosynthetic efficiency, analysed by chlorophyll fluorescence, chlorophyll content, leaf functional traits, carbon reserves (i.e. non structural carbohydrates), tree growth and annual growth dynamic, measured by tree ring, are essential to evaluate functionalities and vitality of trees, the resilience of forest species to environmental changes and their recovery after a disturbance and damage. Several of these indicators can be assessed in specific cases of forest disturbances, for specific species or in particular environmental contexts. Some of them can act as markers of physiological damage suffered by plant, highlighting the severity of the damage and the risk of plant death. The extensive forest monitoring newtwork is an essential tool to know the health state of the Italian forests, to assess the entity of possible chages in species composition and community structure and functioning induced by climate impacts and other environmental factors. The adoption of physiological indicators, combined with the traditional ones based on visual assessment of tree condition, would make the monitoring system updated and with a greater efficiency to evaluate and monitor forest health in long time.

## Contaminants of emerging concern and antimicrobial resistance: use of nature-based solutions for a safe civil wastewater reuse

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Nowadays, it is commonly to find in wastewater drugs, used by humans and/or for livestock, or personal products care, both included in the so-called Contaminants of Emerging Concern (CECs), whose concentrations range from ng $L^{-1}$ to ug $L^{-1}$. Among CECs, antibiotics can promote the selection and diffusion of antibiotic resistance bacteria (ARB) and genes (ARGs). Because of their incomplete removal by conventional treatments, CECs, ARB and ARGs are found in the effluents, posing risks to the environment and public health with particular regards to water reuse. For all these reasons, in our study we tested an innovative NBS (Nature-Based Solution), the so-called Constructed Wetlands (CWs), for the secondary treatment of civil wastewater. CWs exploit the natural remediation capability of plants and microorganisms, so CWs represent an eco-friendly solution for the environmental contaminant removal so as to assure the reuse of treated water in compliance with the italian regulatory limits imposed by D.M. 185/03. Moreover, we wanted to test the capability of CWs to reduce CECs (*e.g.,* antibiotics, ARB and ARGs concentrations) as foreseen by the ISTISAN 21/03 report. Specifically, we assayed the microorganism resistance to four different antibiotics commonly used: Ampicillin, Tetracyclines, Vancomycin and Erythromycin. For this purpose, we carried out two independent experiments based on two pilot-scale plant configurations: hybrid CW flow (inverted vertical and horizontal flow – HCW), and vertical CW flow (VCW). The experiment has foreseen: i) physico-chemical (COD, N, Cl, HM, antibiotics) and biological (BOD, coliforms, enterococci) characterization of the influent and effluent wastewater after 48 hours of water treatment; ii) treatment of an aliquot of the effluent with an UV ray irradiation; iii) analysis of the influent wastewater and effluents (after CWs, after UV) through cultural and molecular methods to evaluate the presence of ARB/ARGs and to quantify the four ARGs by real-time PCR; iv) use of the effluents (after CWs or after CWs + UV treatments) to irrigate 72 seedlings of lettuce for two weeks in order to verify the possible transfer of ARB/ARGs to the seedling rhizosphere or to their edible part (leaves). The CWs treatments resulted effective to obtain water of high quality for irrigation purposes. In fact, it was observed a reduction of the main assayed physico-chemical and biological parameters for both used CWs, in compliance with D.M. 185/03. Cultural methods showed a significant decrease of ARB after 48 hours of wastewater treatment in both CWs and their total removal after UV treatment. Moreover, the genes quantification, throughout real-time PCR analysis, carried out after the CWs treatment, alone or in combination with UV irradiation, demonstrated the ARGs removal up to 97% or 99%, respectively. Finally, cultural methods showed the presence of few ARB only in the rhizosphere of the crops irrigated with CWs, whilst they were absent in the lettuce leaves. Contrarily, crops irrigated with water treated by CW+UV showed the absence of ARB in any part of the plant and ARGs, instead, were not detected in all the rhizospheres analyzed probably because of their low amount. The innovative aspect of this study lies in the civil wastewater treatment through CWs, showing the efficacy of both CWs configurations (HCW or VCW) in water reclamation also in the light of CECs elimination and in the consequent reduction of health and environmental risks.

## Salt-stress, effects on the root system of two *Sorghum bicolor* hybrids: morpho-functional, cytohistological and metabolomic analyses

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Nowadays we are experiencing a constant and rapid decrease in crop yield and quality all over the world, something that clashes with the growth of the human population and its demand for food. Salt stress is one of the most prominent abiotic stresses, affecting water and food safety. Over 7% of the world's lands and 20% of the global irrigated land area are currently salt-affected due to inefficient and uncoordinated water use (1). Europe, like the rest of the world, is experiencing desertification, drought, loss of biodiversity, soil erosion and salinization, a rise of sea level, fresh-water scarcity and heat waves. Wheat, maize, rice and barley, the most cultivated European crops, are sensibly affected by these climate stresses. The territories of southern Europe are the most affected. Italy, in particular, is facing a very high yield decline and it has been estimated that a large portion of its coastal areas in the subsoils (from 30 to 100 cm) are salt-affected. This phenomenon has led to an expansion of marginal areas, also known as "less-favored agricultural areas" because of their unfavorable biophysical characteristics that decrease their agricultural potential (2).
*Sorghum bicolour* (L.) Moench is the 5$^{th}$ cereal globally produced, especially in arid and semi-arid regions, because of its natural resistance against salinity, drought and heat and because of the nutritional characteristics of its kernels, which are gluten-free and have a high protein content. Even if sorghum is not usually grown in European countries, Italy is one of the biggest European producers. Sorghum could be a perfect crop species to address the challenges posed by ongoing climate change. It has the unique anatomical leaf adaptations found in C4 plants, making it well-suited for enhancing marginal and altered soils and contributing to the achievement of agroecological sustainability. Indeed, these kind of plants have a higher efficiency of the photosynthetic pathway which reduces photorespiration energy losses due to low water supply, high temperature and intense light and confers fast-growth and high-yield characteristics even in harsh environments (3).
This study, as part of the CN2 AGRITECH PNRR project, is aimed to analyse the effects of salt stress on the root systems of two commercial sorghum hybrids, both provided by Padana Sementi Elette S.r.l., through morpho-functional, cytohistological and metabolomic analyses. The seeds of the two sorghum hybrids certified as tolerant (Tonkawa) and non-tolerant (Bianca) to drought, were cultured *in vitro* for 10 days and exposed to 0, 150 and 300 mM of NaCl. The presence of NaCl at 300 mM reduced seed germination rate and strongly inhibited primary and adventitious root development in germinated seeds of both genotypes and particularly in Tonkawa, which showed a reduction of 80% in its germination rate. NaCl at 150 mM reduced root biomass and altered root system architecture in both hybrids, mainly affecting lateral root formation and development as well as adventitious root elongation. Tonkawa's root biomass showed a higher decrease than Bianca's. Based on these morphological results, cytohistological and metabolomic analyses were carried out only on control and 150 mM NaCl-treated samples. Metabolomic analysis was performed using $^{1}$H-NMR spectroscopy, monodimensional $^{1}$H and bidimensional $^{1}$H- $^{1}$H TOCSY experiments were acquired by a JNM-ECZ600 Spectrometer. Multivariate analyses (PCA, PLS-DA) were performed on the matrix of acquired data. NMR analysis enabled to identify and quantify 33 metabolites.The PCA comparison between control samples (Bianca *vs* Tonkawa) underlined a complete separation of the two groups. PLS-DA analysis showed a higher level of secondary metabolites (Gallic acid, Dhurrin) in Tonkawa compared to Bianca and a higher level of aminoacid precursors of secondary metabolites (Phenylalanine, Tyrosine, Tryptophan) in Bianca compared to Tonkawa. The treatment with NaCl 150mM stimulated a common biomarker (high level of Ethanolamine). In addition, Bianca showed a significant increase in Leucine, Phenylalanine and Proline levels, while Tonkawa showed an increase in 4-Hydroxybenzoate, Acetic Acid and a decrease in GXP levels. In conclusion, these preliminary results show a different pattern of responses of the two genotypes to salt stress. In particular, Bianca genotype seems to be better adapted to higher salinity levels, suggesting that it could be considered for the recovery of marginal agricultural areas affected by high salt levels.

1) G. Ren, P. Yang, J. Cui, Y. Gao, C. Yin, Y. Bai, D. Zhao, J. Chang (2022) Frontiers in Plant Science, 13.
2) H. Ahmadzai, S. Tutundjian, I. Elouafi (2021) Sustainability, 13(16), 8692.
3) M.M.F. Mansour, M.M. Emam, K.H.A. Salama, A.A. Morsy (2021) Planta, 254(2), 24.

## Threats and vulnerabilities of Sardinian wetlands: from environmental to botanical perspective

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Wetlands are one of the most important ecosystems on Earth. Nevertheless, various challenges threaten these ecosystems and disrupt their ecological character. Among these, the effects of human-based threats are more devastating (1). Here are presented efforts that have been made to analyze wetlands threats, main vulnerabilities and how these affect the flora and vegetation. We monitored 500 wetlands around Sardinia (Italy), including different wetland types according to Ramsar Convention definitions. The information in the field was gathered following the "Rapid Assessment" protocol (2) to provide a general overview of a maximum number of sites with limited resources. Data regarding impacts and floristic records were then analyzed.
Sardinian wetlands are similarly threatened to other ecosystems. Main threats include chronic degradation due to pollution from non-point sources (pollution), human activities such as draining or filling wetlands, hydrological and vegetative alterations. We detected a higher concentration of threats in marine and coastal wetlands than in inland or human-made wetlands (regardless of whether they are protected areas or not). These wetland types include lagoons, estuaries and salt marshes, which cover larger areas and are also easy to reach. Most of the investigated wet areas (208 sites) are threatened by vegetative alteration, understood as a change in the structure of vegetation (105), the introduction of exotic plant species (85), changes in species composition (10) and loss of plant diversity (4), followed by pollution (159 sites) and hydrological impacts (124 sites) (Fig 1a-c). As regards the exotic vascular flora, a total of 128 alien species were found in wetlands, accounting for 8,5% of the total plant species. Invasive species were found in 62 wetlands, while the naturalized in 38 and the casual ones in 23 sites. Among them, the Asteraceae species had the highest frequency (210), followed by Poaceae (196), and Fabaceae (148). The most diverse family was Fabaceae with 11 species, while the Myrtaceae with only three different species were recorded in 130 wet sites (Fig 1d). Comparing these data with previous studies (3) additional families have been reported in wetlands such as the Aizoaceae (82 records and 10 species) and the Cactaceae (93 records and 6 species). The Aizoaceae family were traditionally found in xeric ecosystems, but their tolerance to salt marsh habitat makes them tolerant to live in halophytic conditions mainly in the coastal lagoons and estuaries. The case of the Cactaceae can be more related to human direct actions, such as the abandonment of pruning residues. Among hydrophytes, we recorded *Lemna minuta* (9 sites), *Hydrocotyle ranunculoides* (3 sites), *Eichhornia crassipes* (2 sites) and *Azolla filiculoides* (1 site).
Wetland vegetative alteration and plant invasion have substantial and persistent effects on habitat structure (reducing microtopographic heterogeneity), biodiversity (generally reducing numbers of species of plants and consequently animals), and food web functioning (sometimes increasing food supplies, sometimes changing food quality). The results obtained therefore lead us to enhance multidisciplinary research on the ecology and functioning of wetlands, with the aim of planning more effective conservation actions based on knowledge on plant communities.

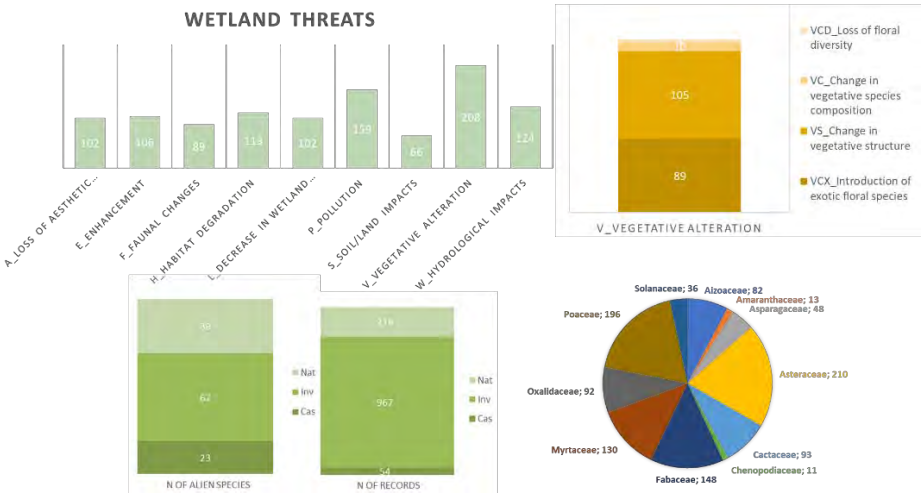


Fig. 1a). Frequency of recorded threats. b). Frequency of different impacts related to vegetative alteration. c). Number of alien species and frequency. d) Number of taxa by families.

1) E. Daryadel, F. Talaei (2014) Int J Environ Eng, 8, 2108-2118.
2) P. Tomàs-Vives (2008) https://medwet.org/codde/wetlandinvetory.html
3) O. Mayoral, F. Mascia, L. Podda, E. Laguna, P. Fraga, et al. (2018) Not Bot Horti Agrobo, 46, 317-326.

## Agrobiodiversity decline and recovery: a multi-scale issue. A pilot assessment experience in the Po Plain district

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The current agrobiodiversity decline trends, its conservation and promotion are a widely recognized urgency. Since the 1960s, the shift towards intensive, highly mechanized agriculture has severely altered the agroecosystem ecological quality and biodiversity values. The agricultural landscape traits result in highly simplified structural and functional patterns. Impacts are generally recognized at field scale, by recording the impairment level of the diversification and quality of rural spontaneous bio-coenoses. Nonetheless, farm scale, local and extra-local scale dynamics directly and indirectly influence field scale bio-coenoses structural, functional and dynamic traits (1). Hence, multi-scale biodiversity monitoring approaches are needed, to coherently understand and address agrobiodiversity issues. This is especially true in intensively anthropized landscape systems, such as the agricultural ones, where the landscape eco-mosaic health status is significantly affected by human activities. In line with these acknowledgements, we are conducting a study in the Po Plain western district, a highly anthropized region currently experiencing significant impacts on biodiversity, paired to a spread depletion of natural habitats. The study aims at identifying suitable tools for agrobiodiversity monitoring, by testing and comparing multi-level analytical tools. Landscape ecology analyses are led at different scales of analysis, with different levels of detail (2). Quantitative metrics are computed and compared (patch size, matrix, shape indices, diversity and connectivity indices, a landscape metastability index). A newly adapted connectivity index is tested, synthetically accounting for the ecological quality of corridors (development, stratification, continuity and allochthonous degree). Current state and transformation scenarios are evaluated. Results are then crossed with field and farm scale floristic-vegetational analyses (3), to identify significant correlation patterns. We here present the first results on landscape ecology analyses, applied to a pilot farm (Vercelli district) adopting an agroforestry approach, which is compared to the conventional surrounding agricultural settings. These analyses allowed us to account for the positive contributions given by the agroforestry approach towards higher values of landscape diversification, rebalancing the landscape eco-mosaic composition, if compared to farm scale and local scale conventional management. The ongoing multi-disciplinary study of the area allowed us to identify specific design interventions on rural landscape features rehabilitation, both in coherence with the local floristic-vegetational patterns and with the outlined landscape eco-mosaic shortcomings. Hence, transformation scenarios were assessed through connectivity analyses, quantifying the potential positive contribution given by the agroforestry management model expansion. Such results frame the ongoing evaluation of field scale floristic-vegetational qualitative and quantitative traits. This allows the building of an analytical framework for representing both the current agrobiodiversity traits, the reasons behind their negative trends, and the multi-scale factors influencing such patterns, also allowing to display the underlying delivering of Ecosystem Services and Disservices. This approach is intended to inform both farmers, public administrations, and agricultural policies, by orienting them towards targeted interventions for effectively addressing biodiversity issues.

1) W.E. Dramstad, J.D. Olson, R.T.T. Forman (1996) Island Press, 1-80.
2) I. Vagge, G. Chiaffarelli (2023) Agronomy, 13, 233 https://doi.org/10.3390/agronomy13010233
3) I. Vagge, G. Chiaffarelli (2023) Plants 2023, 12, 2012 https://doi.org/10.3390/plants12102012

## The mycorrhizal root-shoot axis elicits *Coffea arabica* growth under low phosphate conditions

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Coffee (*Coffea* sp.) is among the most traded food products worldwide with an increasing consumption trend which has been estimated to reach an annual growth rate of 4.28% in 2022-2027 period. However, in the context of global climate changes coffee production is threatened (1) with an estimated 50% reduction in lands suitable for its cultivation in the next few decades. Like 70% of land plants, *Coffea* species associates with arbuscular mycorrhizal fungi leading to a strong mycorrhizal-induced growth effect with slight variations in different genotypes (2). However, even if a number of studies investigated agro-management effects on coffee root colonization under field conditions (3), the underlying physiological and molecular bases of such phenotype still remains fully unknown.

In this study, we used an integrative multi-omics approach to analyze the effects of mycorrhizal inoculation in two *Coffea arabica* genotypes ('Typica National' and 'Catimor Amarillo') using *Funneliformis mosseae*.

Both the studied genotypes, even if allowing a relatively low level of mycorrhizal colonization, showed a significant biomass increase upon inoculation. Most of the membrane transporter homologues already characterized in other model species were activated in the transcriptome upon colonization. Being this non-model species allo-tetraploid, many of these transporters including the mycorrhizal-induced phosphate and ammonium transporters, occurred in different genome copies, namely the homeologs originating from the *C. arabica* parentals (*C. canephora* and *C. eugenioides*). Such transcriptional responses were validated using metabolome profiling and physiological measurements. Data showed higher amounts of fructose, glucose, flavonoid glycosides in mycorrhizal plants. Interestingly, we found the up-regulation of the phenylpropanoid pathway, which however did not lead to an increased lignin synthesis as happens in other plant models. Though, metabolomics showed that this pathway was deviated towards the synthesis of glycosylated flavonoids which share many upstream intermediates with the lignin synthesis. Moreover, the integration of physiological and molecular data showed a higher efficiency of photosystems functioning and an enhanced $CO_2$ assimilation in mycorrhizal plants, independently of the plant hydraulic regulations. Overall, the effects of arbuscular mycorrhizal colonization on both coffee cultivars revealed a deep re-organization of the major metabolic routes, from nutrient acquisition, to carbon fixation and secondary metabolism following the root-shoot axis.

Our study provides the first molecular evidence of the mycorrhizal-induced growth response in *C. arabica* showing how the integration of multiple 'omics' can enhance our comprehension of symbiotic interactions in non-model plant species. In the case of coffee this paves the way for potential applications in coffee nurseries where the need to improve plant health and survival is a rising demand.

1) M. Adhikari, E.L. Isaac, R.R.M. Paterson, M.A. Maslin (2020) Microorganisms 8, 1625.
2) A.J. Fonseca, A.F. Freitas, G.R. Carvalho, M.A.C. Carneiro, D.J.M. Vilela, L. Fassio (2019) Ciência e Agrotecnologia 43.
3) S.R.D. Aguila SR-D, A.M. de la Sota-Ricaldi, M.A. Corazon-Guivin, Á. López-García (2022) Journal of Soil Science and Plant Nutrition 22, 3291–3303.

## Olive oil exhausted pomace Green extraction techniques: phytochemical characterization and biological activities

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The cultivation of olive trees (*Olea europaea* L.) and olive oil production represents a focal point around which the Mediterranean countries' cultural and economic life was developed. World olive oil production is around 3.32 million tons per year, with Italy (17%) among the leading producers (1, 2); from this, the problem of management of olive mill by-products, including wastewater and solid residues, often called olive pomace. The first pomace, known as 'virgin', derives from the first olive press and is often exploited for pomace oil production through hexane extraction. Once dried, this last pomace, called 'exhausted', represents an excellent solid biofuel employable in industrial boilers and for electric energy generation. However, exhausted olive pomace is also known for being rich in secondary metabolites, hence the need to extract them. This is often made using conventional methodologies involving toxic organic solvents. However, the major limits of these techniques are the large consumption and evaporation of solvents, longer times, low selectivity, and thermal degradation of compounds. Thus, in recent years, alternative extraction techniques have aroused particular interest. Among them, microwaves, ultrasounds, and pressurized liquid extraction are the most advantageous in terms of sustainability. Although there are many studies about optimizing these methods on virgin olive cakes, leaves, and wastewaters, few articles have focused on the phytochemical extraction, characterization, and antioxidant activity of exhausted pomace. Hence, in view of its nutraceutical valorization, conventional and emerging extractive technologies were compared using green solvents (3). Specifically, 4 different extractive techniques [Maceration (ME), Ultrasound Assisted Extraction (US), Microwave Assisted Extraction (MW), and Accelerated Solvent Extraction (ASE)] and two different solvents (Water and 50% Ethanol/Water) were employed. Extracts were preliminarily tested for their antioxidant and hypoglycaemic activity using cell-free assays. Specifically, 4 complementary antioxidant assay was used: the Total Phenolic Content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), and $\beta$-Carotene Bleaching assay (BCB). The best results in terms of antioxidant activity were obtained using the FRAP assay reaching values from 86.51 ± 8.68 to 112.16 ± 1.20 mg TE/g and 107.13 ± 8.68 to 129.95 ± 10.31 mg TE/g for aqueous and hydroalcoholic extracts, respectively. Furthermore, to compare the data obtained from all the antioxidant tests used, the Relative Antioxidant Capacity Index (RACI) was determined. The RACI is an adimensional and statistical index computed using Excel software (2010, Microsoft, Redmond, WA, USA) by integrating the value obtained from the evaluation of antioxidant activity with the different assays. Using this index, it was demonstrated that extracts made with ASE and US displayed the highest antioxidant activity. On the other hand, the hypoglycaemic activity was assessed through the $\alpha$-amylase and $\alpha$-glucosidase assays even if, in this case, no hypoglycaemic activity was seen in all cases. Hence, based on the result from RACI, hydroalcoholic ASE and US extracts were tested on HepG2 used as cellular model, demonstrating no cytotoxic effect, but their ability to restore ROS levels to the basal state after stress induction was measured. Considering these promising results, the ASE and US hydroalcoholic extracts were characterized by HPLC-DAD analysis. This allowed to identify and quantify, in the pomace extracts, 7 polyphenolic compounds at 280 nm, according to standards retention times and UV-spectra: 3,4-dihydroxybenzoic acid, 3-hydroxytyrosol, gallic acid, catechin, tyrosol, vanillic acid, and, for the first time, salidroside. Among the identified compounds, the 3,4-dihydroxybenzoic acid is the most abundant (14.54 ± 0.28 and 14.31 ± 1.07 mg/g for US and ASE, respectively), followed by 3-hydroxytyrosol and salidroside. In conclusion, this study demonstrated that the best extractive techniques to valorize exhausted olive pomace are ASE and US obtaining extracts with the highest antioxidant capacity and the greatest amount of phenolic compounds known for exerting several biological activities. Furthermore, the absence of cytotoxicity opens great possibilities for applying these extracts in the nutraceutical field, transforming a waste product into a rich source of specialized metabolites in a circular economy perspective.

1) V. Lo Giudice, I. Faraone, M. R. Bruno, M. Ponticelli, F. Labanca, D. Bisaccia, C. Massarelli, L. Milella, L. Todaro, (2021) Molecules, 26(16), 5081.
2) A. Khdair, G. Abu-Rumman, (2020) Processes, 8(6), 671.
3) D. Russo, I. Faraone, F. Labanca, C. Sinisgalli, M. Bartolo, P. B. Andrade, P. Valentao, L. Milella, (2019) Phytochemical Analysis, 30(5), 547-555.

**Simultaneous characterisation of *Prunus persica* fruit volatilome and gene expression profile for a better understanding of molecular mechanisms underlying changes of peach during post-harvest storage**

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Fruit quality is determined by numerous traits including the phytochemical composition which determines aroma, flavour and sweetness among other characteristics relevant for the consumer. Peach fruits are characterized by a rapid deterioration at room temperature meaning that cold storage is widely used to delay post-harvest ripening of the fruit and extend its commercial life. It is, therefore of considerable scientific and economic interest to improve our knowledge of the mechanisms by which fruit respond to cold stress. Volatilome fingerprinting combined with gene expression profiling can provide evidence for fruit quality differences and changes which can be related to genotype selection, geographical origins, post-harvest storage and supply chain processing (such as washing, drying, and trimming). In this context, next generation sequencing and metabolomics technologies can be very useful by allowing comprehensive, simultaneous characterization of metabolite and gene expression data from diverse genotypes of the same species as well as assessing effects of post-harvest storage conditions.
Here, we combined analysis of the volatilome of peach (*Prunus persica* L. Batsch) fruits using comprehensive two-dimensional gas chromatography (GC×GC) combined with time-of-flight mass spectrometry (TOF-MS) with identification of differentially expressed genes (DEGs) associated with volatile organic compound (VOC) metabolism using transcriptomics during post-harvest treatments to better understand mechanisms underlying post-harvest processes.
Our study, focussed on one peach (cv Sagittaria) and one nectarine (cv Big Top) cultivar: fruits were analysed immediately after harvest and after 1, 5, 7 and 14 days of cold storage at 1°C.
A total of 159 VOCs were identified for Sagittaria, while 89 VOCs were detected for Big Top. Canonical Analysis of Principal coordinates (CAP) of VOC profiles showed a discrimination between cultivars and post-harvest storage periods. Furthermore, expression profiles of VOCs-related genes correlated with VOCs. For example, in Sagittaria we observed a genotype specific activation of VOC biosynthetic pathways related to sesquiterpenoid and triterpenoids biosynthesis.
Overall, the combination of VOC profiles and gene expression could help breeders to understand which traits/aroma are more relevant to consumer perception. Furthermore, understanding of metabolic and genetic changes occurring in fruit VOC patterns post-harvest could provide a suite of simple diagnostic checks to monitor fruit quality throughout the supply chain.

## Wonder, Power, and Love: Agostino Chigi's message written in the language of plants at the dawn of a new era

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At the beginning of the Modern Era, the "Magnus Mercator" Agostino Chigi commissioned Raphael to design the frescoes in the Psyche's Lodge at the entrance of his private residence in Rome (later named as Villa Farnesina). A frame of hanging garlands runs along the edge of a mythological scene onto the ceiling of the Lodge, where the botanical elements were painted by Giovanni da Udine, a member of Raphael's workshop who specialized in the depiction of plants and natural features. Around 170 different species can be identified in the frescoes, and this botanical variety never seen in a single work of art testifies the artist's profound knowledge of the natural world. Furthermore, the botanical decorations collect about 1,200 separate elements, and if individual flowers and fruits are counted, the total rises to several thousand [1,2]. The sense of wonder inspired by the garlands is in part due to the sensation of finding oneself presented with all that nature has to offer or, in the words of Vasari, with a documentation of "all sorts of fruits, flowers and leaves, season by season". This sensation is greatly reinforced by the sight of rare species and of plants that were completely unknown to the European observer, having only just arrived from the Americas, increasing the garlands' impact as instruments of wonder, and inspiring the visitor's admiration for the splendour of the villa and for the power of its 'Magnificent' owner. A prominent role belongs to the theme of love, as shown by the numerous references to the goddess Venus, such as many varieties of apples and roses, but also by flowers and fruits associated with the goddess Juno, such as lilies, jasmine, pears and quinces, and hesperidia connected to the myth of the Garden of Hesperides. Overall, the frescoes have a consciously innovative style departures from the aesthetics of the time, and the exceptionally rich diversity of plants found in the frescoes can be seen as a case-study of contemporary knowledge and use of plants, indicating which species were used for food, medicine, or as ornamentation, and which wild or cultivated plants were known at the time. Despite this abundance of plant life, what makes this work unique not only in Italy but for the whole of Europe is that the paintings include the earliest depictions of plants introduced to Europe from the Americas, only twenty years after Columbus rediscovered the continent.


1) Caneva G. (1992) Il mondo di Cerere nella Loggia di Psiche, Ed. Palombi, Roma: pp. 1-223. ISBN 9788876217777
2) Caneva G. (2022) Meraviglia, amore e potere: le pitture botaniche più ricche del mondo nella Loggia di Psiche di Raffaello e Giovanni da Udine: Villa La Farnesina, Roma Ed. Nardini Firenze. COD: 9788840406220-1

## In vitro digestion of three Ethiopian varieties of *Eragrostis tef*

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This study is part of a program funded by the Italian Agency for Development Cooperation, known as 'Seeds of the Future', which aims to encourage a healthy and appropriate diet in order to prevent and address malnutrition among young children and breastfeeding mothers in a specific region of Ethiopia. Teff (*Eragrostis tef* (Zucc.) Trotter) is a fundamental component of the Ethiopian diet, renowned for its high concentration of polyphenols, particularly flavonoids. These compounds have demonstrated significant antioxidant properties, which effectively complement the effects of vitamins and enzymes in minimising oxidative stress in cells that are subjected to malnutrition. It has been observed that malnutrition can trigger the generation of radical species, including the superoxide anion $O_2$-, hydroxyl radical OH-, and hydrogen peroxide $H_2O_2$ [1]. There are two potential causes for the observed effects, one being the accumulation of reactive oxygen species (ROS), which results from a decreased intake of various essential nutrients, such as carbohydrates, proteins and vitamins. The other potential cause is chronic inflammation, which results in non-specific and long-term activation of the immune system. An assessment was conducted on three distinct indigenous teff varieties, namely Red, Debrezeit, and Quncho, which were gathered from diverse parts of the project region. The evaluation was based on their flavonoid content using RP-HPLC-DAD-MS, as well as their antioxidant activity utilising the DPPH test. Starting from the findings obtained, it has been confirmed that brown teff contains a higher concentration of luteolin derivatives, while white teff contains a higher concentration of apigenin derivatives. These results align with existing literature reports and provide valuable insights into the composition of these two teff varieties [2]. The crude drugs underwent *in vitro* digestion [3] in order to assess the release of flavonoids present within the caryopsis and to determine whether they remained unaltered until intestinal absorption. The findings of our study indicate that gastrointestinal fluids have an extraction efficiency of 80% total flavonoids. The impact of the digestion process varies based on the type of teff being examined. White teff (Quncho) demonstrated the highest percentage of extracted flavonoids in the gastric phase at 63.14%. Conversely, the mixed (Debrezeit) and brown (Red) varieties showed the most significant effect in the intestinal phase, with percentages of 57.35% and 58.14%, respectively. Our research found that the Debrezeit variety had the highest intake of flavonoids in the intestine, providing 101.25 mg/100 g of dried teff in the diet. Furthermore, this variety exhibited the highest radical scavenger capacity in the DPPH test. We compared the antioxidant capacity of the pre-digestion extract with the post-digestion residue and found that the latter had approximately seven times less activity. This depletion of the matrix resulted from the *in vitro* process and was confirmed through RP-HPLC-DAD analysis of the total flavonoid content. These findings suggest that teff can be considered a strategic cereal in the fight against malnutrition and provide an encouraging contribution to the ongoing efforts to combat this global challenge.

1) R.A. Ghone (2013) J. Clin. Diagn. Res. 2013. doi:10.7860/JCDR/2013/6019.3454.
2) S. Ravisankar, K. Abegaz, J.M. Awika (2018) Food Chem., 263, 265–274. doi:10.1016/j.foodchem.2018.05.002.
3) M. Minekus, M. Alminger, P. Alvito, S. Ballance, T. Bohn, C. Bourlieu, F. Carrière, R. Boutrou, M. Corredig, D. Dupont, et al. (2014) Food Funct, 5, 1113–1124. doi:10.1039/C3FO60702J.

## Metabolomic analysis and bioactivities of *Arbutus unedo* leaves harvested across the seasons in different natural habitats of Sardinia (Italy)

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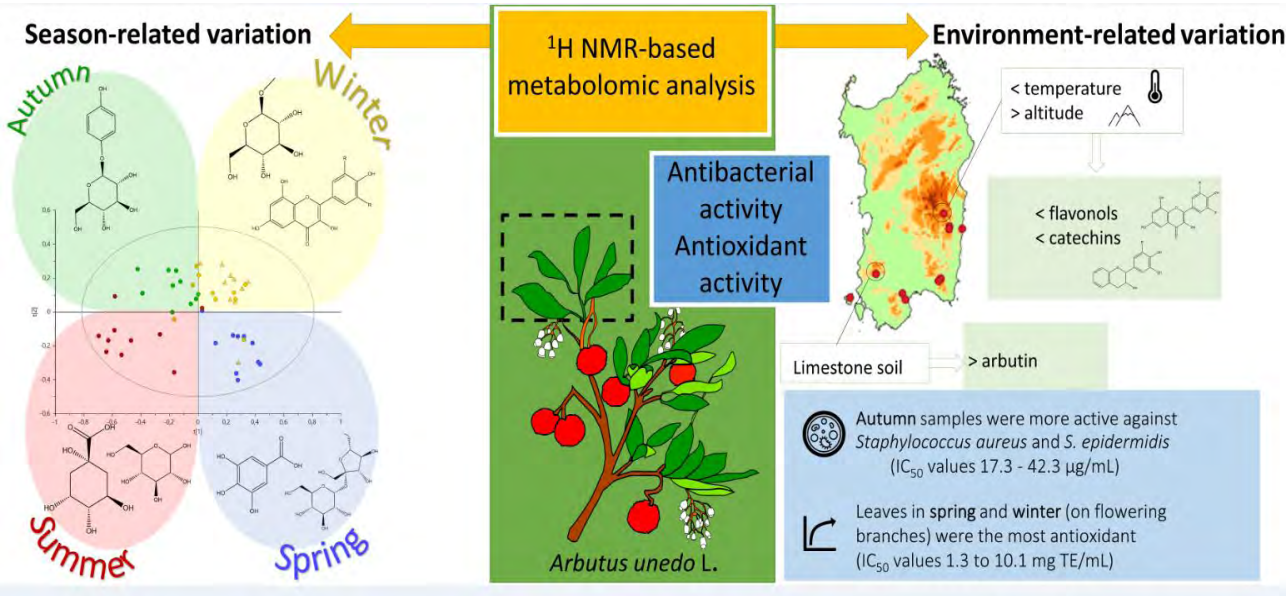
*Arbutus unedo* L. is a wild tree of Mediterranean regions used as food and in traditional medicine and important for afforestation programs. There is no detailed information available on the variation of *A. unedo* leaves metabolome across the seasons. The leaves (Fig.1) were analyzed by $^1$H NMR-based metabolomics, comparing samples harvested across the seasons and in ten different natural habitats of Sardinia (Italy) (Fig.2).
Results Multivariate analysis showed the impact of seasonal variation on the metabolome: glucose and quinic acid increased in summer, while in spring sucrose was accumulated. β-Arbutin, the main known active principle of *A. unedo*, generally reached the highest concentration in autumn. In winter, O-β-methylglucose, GABA, flavonols (quercetin-3-O-α-rhamnoside, myricetin-3-O-α-rhamnoside, kaempferol-3-O-α-rhamnoside), catechin, and gallocatechin increased. Characteristic metabolomic features were found also for samples collected in different locations. For instance, trees growing at the highest altitude and exposed to lower temperatures produced less flavonols and catechins. The only samples collected on trees growing on limestones, dolomites, and dolomitic limestones type of soil showed generally the highest content of arbutin. The highest phenolics content was found during spring, while samples collected on flowering branches in winter were the ones with the highest flavonoid content. The antioxidant activity was also variated, ranging from 1.3 to 10.1 mg TE/mL of extract, and it was positively correlated to both total phenolics and flavonoid content. Winter samples showed the lowest antibacterial activity, while summer and autumn ones exhibited the highest activity ($IC_{50}$ values ranging from 17.3 and 42.3 µg/mL against *Staphylococcal* species).
In conclusion this work provides for the first time $^1$H-NMR fingerprinting of *A. unedo* leaves, elucidating the main metabolites and their variations during seasons. On the basis of arbutin content, autumn could be considered the balsamic period of this taxon. Samples collected in this season were also the most active ones as antibacterial. Moreover, an interesting metabolomic profile enriched in catechins and flavonols was observed in leaves collected in winter on flowering branches which were endowed with high antioxidant potential.



## Phytochemical investigation of *Cornus sanguinea*

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*Cornus sanguinea* L. (Fig.1), belonging to the Cornaceae family, is widespread in Central Europe and in the Mediterranean area. Species of the genus *Cornus*, widely used in traditional and modern medicine, are very rich in phenolic compounds. The leaves and fruits possess antioxidant, anti-inflammatory, cytoprotective, analgesic, antidiabetic and anticoagulant activities (1). Recent observations showed how the presence of *Cornus sanguinea* L. close to the hazelnut cultivation (*Corylus avellana* L.) could be able to limit the attack of hazelnuts by the balanine pathogen (*Curculio nucum* L.) (2). With the aim to highlight the compounds responsible for this activity, a phytochemical investigation of the methanol extract of the whole dried plant of *Cornus sanguinea L.* was carried out.
An initial LC-MS profile of the MeOH extract of *C. sanguinea* (Fig.2) was performed by UPLC-Q-Exactive-Orbitrap MS/MS and it guided the isolation of compounds, the structures of which were unambiguously elucidated by NMR analysis as phenolic and terpene derivatives. Among these, one compound belonging to the megastigmane class was described for the first time. Further analysis was performed on the dried leaves of *Cornus sanguinea* L. by HR-MAS NMR (High Resolution Magic Angle Spinning NMR Spectroscopy), leading to the identification and quantitative analysis of primary metabolites.
LC-MS profile along with HR-MAS NMR analysis allowed to obtain a deep insight on *C. sanguinea* metabolome, creating the basis to further investigate the compounds responsible to protect *C. avellana* from the balanine attack.

Fig.1. *Cornus sanguinea* L.

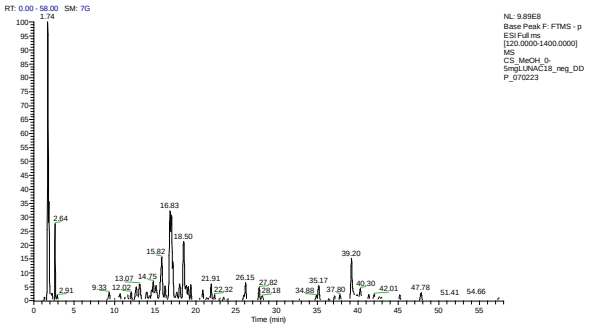


Fig.2. LC-MS profile of *Cornus sanguinea* L. methanolic extract.

1) M.S. Stanković, M.D Topuzović (2012) Acta Botanica Gallica: Botany Letters, 159(1),79–83.
2) X. Li, D. Xiu, J. Huang, Yu B., S. Jia, L. Song (2023) Forests, 14, 319.

## Multifaceted assessment of vegetation and soil temperatures changes in the Orobic Alps after 13 years of monitoring

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Rising temperatures in alpine regions are leading to an increment in species richness due to upward migration of new plant species from lower elevetion, alongside local extinction or population shrinkage of endemic and cryophilc species, severely menaced by both climate change and competition.
Here, we compare the vegetation in two resurveys (2009 and 2022) performed in the Orobic Alps GLORIA (GLobal Observation research Initiative in Alpine environment) study site, alongside microclimatic data. The study site comprehends four low elevation alpine summits at the southern outskirt of the Italian alps, that act as a first sentinel for monitoring change. In order to give a multi faceted assessment of the vegetation change in the study site, we firstly assessed the species that increased and decreased the most by the means of "Cliff" effect size, also checking for local extinctions and colonizations. We assessed changes in species richness, diversity (Diversity profiles defined by Hill numbers) and Turnover. Subsequently, throught the Landolt ecological indicators, we assessed eventual thermophilization and nitrophilization of the study site, alongside changes in the abundance of growth forms and chorotypes through linear models.
After 13 years, average summer soil temperature increased significantly, while snow cover period length decreased over time. Several new species colonized the site, while only few species disappeared. As a consequence, species richness increased. We registered a significant increment of thermophilic and nitrophilous species in the highest summits, and of eurasiatic and mediterranean species overall, while arctic alpine species became less frequent. Alongside specie increment, few common species became more abundant and dominant in the community: diversity profiles became steeper, underling a change in the plant community structure and diversity. Moreover, the summits were characterized by a high level of species turnover, highlighting a fast change in plant communities' composition as a response to climate change.

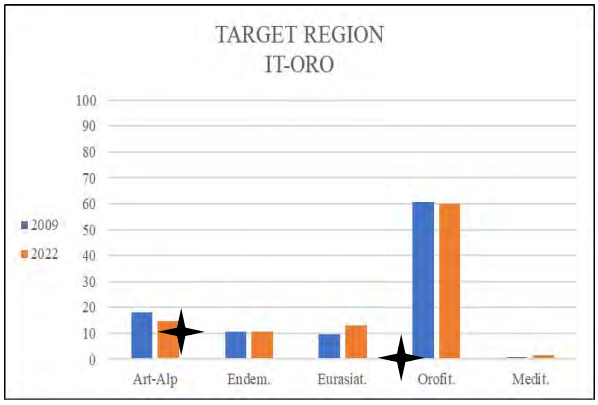


Fig.1. Chorotype changes in IT ORO.

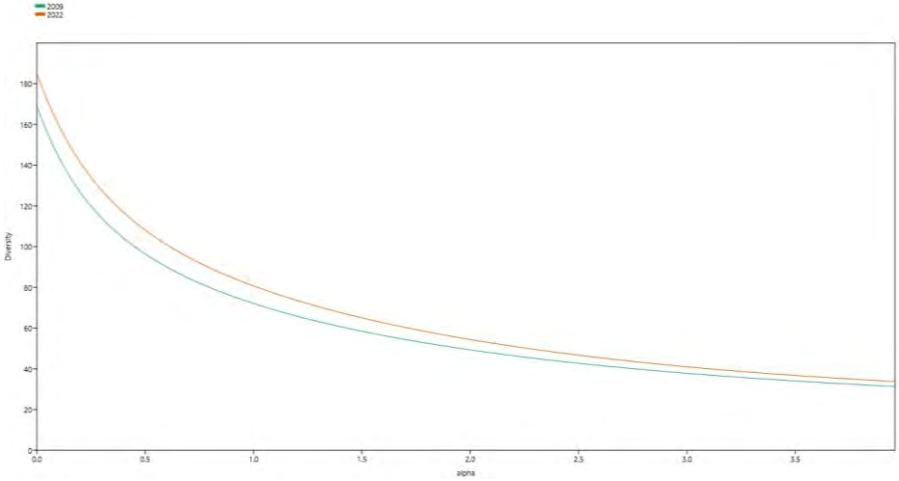

Fig. 2. Diversity profile computed by Hill's numbers. The upper orange profile shows higher diversity level during year 2022 than the lower green profile.

## Vegetation dynamics and species responses of an alpine grassland to 5 years of experimental warming and drought

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The last decade has been characterized by some of the warmest and driest years on record (1). Alpine ecosystems, in particular, are already experiencing major increases in temperature (2), with summer precipitation also predicted to decrease in some areas of the Alps by the end of the current century (3). Research is, therefore, needed to understand how such changes will affect alpine plant communities, for which few studies are yet available, especially concerning responses to reduced precipitation.
With the current research, we aim to investigate vegetation responses of an alpine *Carex curvula* grassland to experimental warming and drought carried out during the growing season in the last 5 years (2018-2022). The main objectives of the present study are to: 1) Assess whether the alpine grassland is experiencing short-term vegetation changes under current climate conditions; 2) Determine if vegetation dynamics will be affected by future warming and drought scenarios; 3) Identify those species which are more sensitive to altered climate conditions. Analysis was performed on vascular species, mosses and lichens cover data visually estimated each year in 20 permanent plots assigned to one of the following treatments (5 replicates): ambient control, warming with open top chambers, precipitation reduction with partial rain-out shelters, and combined warming and drought.
Small, but significant species cover variation was found both over time and between treatments, with climatically manipulated plots experiencing grater shifts in species composition compared to controls. At the species level, however, both increases and decreases in cover were observed, with no effect of either experimental warming or precipitation reduction (singly or combined) on the dominant sedge. Among the most frequent species, only *Phyteuma hemisphaericum* showed an increasing trend in in warmed plots compared to controls. Over time, major reductions in cover were observed for snowbed specialists (e.g. *Soldanella pusilla*). Results allow us to conclude that a variation of the floristic assemblage of the target alpine grassland is already detectable in the short term and could be accelerated by treatments simulating future changes in temperature and precipitation. Drought and warming could interact, altering the performance of some species, while a general decline in the most cold-adapted taxa seems likely to occur in the future.

1) World Meteorological Association (2023) WMO, 1316, 1-49.
2) N. Pepin, R.S. Bradley, H.F. Diaz, M. Baraër, E.B. Caceres, N. Forsythe, H. Fowler, G. Greenwood, M.Z. Hashmi, X.D. Liu, J.R. Miller (2015) Nat. Clim. Change, 5, 424-430.
3) A. Gobiet, S. Kotlarski, M. Beniston, G. Heinrich, J. Rajczak, M. Stoffel (2014) Sci. Total Environ., 493, 1138-1151.

## Surveying pasture communities in diachronic analyses by 3D Models

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Primary productivity is a robust indicator of ecosystem functioning because of its close relationships with the stability of the ecological systems. In ecological research, above-ground biomass (AGB) is the most commonly used proxy of primary productivity. Destructive AGB measurements, although accurate, are time-consuming and do not allow for repeated measurements as required by monitoring protocols. Structure-from-motion (SfM) photogrammetry has been proven to be a reliable tool for rapid and not destructive AGB estimations in grass systems. Three-dimensional (3D) models of 1 x 1 m2 pasture plots were reconstructed (Fig. 1) and AGB volume was measured under several measurement settings and based on four different predictors (height, volume, volume adjusted, and cover volume), in order to evaluate their robustness against within- and between-community heterogeneity. Volume-based AGB measures were regressed to AGB values resulting from destructive methods to identify the measurement settings that show the best fit. Furthermore, 3D models of four mountain pasture plots were reconstructed in May, July, and August. Models relative to the same plot were aligned and their relative difference was measured to produce a diachronic canopy variation model (DCVM) (Fig. 2). The measurement setting for AGB volume estimations strongly influenced their correlation with traditional AGB scores. The best fit was obtained by selecting 1 mm grid cell size and minimum point height distance and applying the adjusted volume values (Vadj). Our study indicates that image-based photogrammetric techniques allow for reliable non-destructive measurements of surface biomass in diachronic analyses, offering a valuable tool for evaluating occurrence, magnitude, and spatial patterns of variations of community primary productivity over time. Diachronic canopy variation model produced congruent patterns of inter-seasonal canopy variations proving to be a useful tool for analysing local disturbance to vegetation canopy caused by grazing.

Fig. 1. 3D model of a pasture plot.

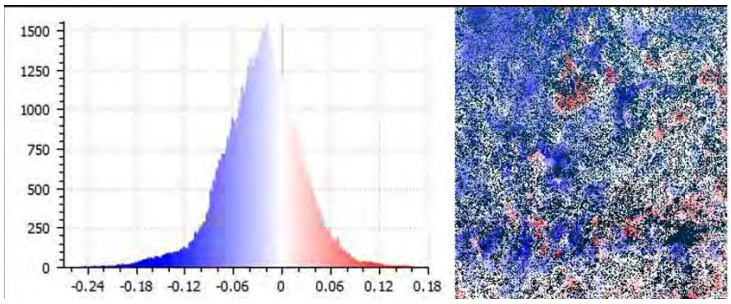


Fig. 2. Diachronic canopy variation model (DCVM).

## Coordination between leaf and root traits in Mediterranean coastal dune plants

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The global spectrum of plant form and function allows to understand plant performance, species distributions, and ecosystem processes worldwide. Whilst most research has focused on aboveground vegetative traits (e.g., leaf economic spectrum, LES), contrasting evidence on any coordination between the LES and RES (root economic spectrum) has been reported, depending on the study system, considered traits or phylogenetic constraints (1). As traits covariation under strong environmental pressures could exhibit multidimensional pattern, studying how different functions (and related traits) vary along environmental gradients and accounting for species' phylogenetic relatedness may help to elucidate the strength of coordination between above- and belowground trait variation.
Here, we tested for a coordination between leaf and root traits within a phylogenetic comparative framework, by focusing on 39 angiosperm species living on coastal dunes of central Mediterranean. Plants were sampled in 3 distinct habitats aligned along the shoreline-inland gradient: (i) "front-habitat", located between the shoreline and the foredune crest, exhibiting the most extreme environmental conditions (substrate instability, salinity and water stress); (ii) "back-habitat", located behind the foredune crest in the interdunal grasslands and the fixed dunes, characterized by higher soil stability, low levels of salinity, but subjected to water stress; (iii) "slack-habitat", located behind the foredune crest in the dune slacks that were occasionally flooded, characterized by higher substrate stability, and low levels of salinity (2).
Three-quarter of trait variation is captured in two-dimensional spectra with low phylogenetic signals, indicating coordination and trade-off between leaf and root traits only moderately influenced by the species' phylogenetic relatedness. Specifically, each habitat spectrum is shaped by the interaction of the carbon economic strategy and the resistance to several environmental conditions that change along the ecological gradient. Indeed, the low phylogenetic contribution in each habitat is likely driven by the strong environmental pressures acting as trait-filtering. Moreover, aboveground traits support the LES in all habitats, while belowground traits are consistent with the RES in the back-habitat only. A coordination between leaf and root traits was found in the back-habitat, which is characterized by less environmental pressures, supporting the whole-plant spectrum (PES). No coordination was found in the other two habitats, facing more extreme environmental conditions (front-habitat) or strong fluctuation of water availability during the year (slack-habitat).
This study confirms the complexity to search for a PES in ecosystems characterized by multiple environmental pressures as those investigated here. Exploring functional diversity of both above- and belowground traits at the community level and accounting for regeneration traits (3) within a phylogenetic framework may be a future perspective to disentangle functional (co)variation in our study system.

1) M. Weemstra, L. Mommer, E.J.W. Visser, et al. (2016) New Phytologist, 211, 1159-1169.
2) D. Ciccarelli, C. Bona (2022) Estuaries and Coasts, 45, 114-129.
3) A. Carta et al. (2022) Annals of Botany, 129, 775-786.

## LC-MS untargeted metabolomic analysis of the halophyte *Salsola tragus* in the climate change scenario revealed a promising phytocomplex

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Climate change causes higher temperatures, raised sea levels, and altered precipitation patterns. These phenomena influence with each other and lead to a vicious circle with many harmful environmental implications, including an increased soils salinization that determines serious consequences for agriculture, native vegetation, and ecosystems in general (1). It affects the plant growth by negatively influencing the nutrient uptake and by causing water stress. In this context, the interest in halophyte plants is significantly growing up. Halophytes are plant species suitable for living in environments with high concentrations of salinity, such as coastal areas or brackish basins, since they developed special mechanisms to tolerate salty soils or water which make them able to survive in environments where many other species would not be able to thrive (1).
The genus *Salsola,* belonging to the family Amaranthaceae, includes about 120 halophyte species of herbaceous or shrubby plants, widespread especially in the brackish grounds of the moderate and subtropical regions of Europe, Asia, Africa, and North America (2,3). *Salsola tragus* L., commonly known as Russian thistle or tumbleweed, is a highly adaptable and fast-growing annual plant. It has an upright appearance and can reach a height between 30-120 cm. Its leaves are small, green, red, or striped with a cylindrical shape and a pointed tip. They are arranged alternately along the stem. The flowers are small, greenish or pinkish.
*Salsola* species are well-known in folk Chinese and Russian medicine as anti-hypertensive, diuretic, anti-cancer, emollient, purgative, anti-ulcer, and anti-inflammatory plants. In continuing our study on halophyte plants belonging to *Salsola* genus (2), *Salsola tragus* L. was herein investigated. Despite its ecological resilience and ancient popular uses, few phytochemical studies on *Salsola tragus* L. are reported in the literature. Tetrahydroisoquinoline alkaloids (salsoline and salsolidine), fatty acids, and flavonoids (isorhamnetin derivatives) were previously found as constituents of this halophyte and its extracts were tested for the antioxidant activity (3).
Tuscan coasts are rich in *Salsola tragus* L. plants, which were collected with the aim of carrying out for the first time an untargeted metabolomic study by UHPLC-HR-Orbitrap/ESI-MS analysis on the extracted phytocomplex. The raw material was dried in the oven at 40 °C, defatted with *n*-hexane, and subjected to dynamic maceration with methanol for three consecutive days. The methanol residue was injected into the LC-MS system for the chemical fingerprint study. A total of 15 compounds was tentatively identified by comparing retention times, HR full mass spectra, and fragmentation patterns with data reported in the literature and database, and considering a mass error < 5 ppm on the experimental molecular formula. The identified components were mainly quercetin, kaempferol and isorhamnetin mono/diglycosides, together with phenethylamine alkaloids (*N*-feruloyltyramine and *N*-feruloylmethoxydopamine) and fatty acids (malyngic acid, pinellic acid, and linoleic acid).
In conclusion, qualitative analysis showed the occurrence of many flavonoid derivatives with proven health-promoting properties and a varied chemical profile. Particularly noteworthy are the two identified alkaloids which are reported in the literature for the anti-inflammatory effect and protective role against β-amyloid peptide-induced neurotoxicity. *Salsola tragus* L., thanks to its remarkable environment adaptability and chemical composition, is an interesting biomass from a botanical, agricultural and nutraceutical point of view encouraging future studies on its potential biological value.

1) T.J. Flowers, A. Muscolo (2015) AoB Plants, 7, plv020.
2) A.M. Iannuzzi, R. Moschini, M. De Leo, C. Pineschi, F. Balestri, M. Cappiello, A. Braca, A. Del-Corso (2020) Food Biosci., 37, 100713.
3) M.R. Loizzo, R. Tundis, G. A. Statti, N.G. Passalacqua, L. Peruzzi, F. Menichini (2007) Nat. Prod. Res., 21, 846-851.

## Soil seed bank responses to edge effects in temperate European forests

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The amount of forest edges is increasing globally due to forest fragmentation and land-use changes. However, edge effects on the soil seed bank of temperate forests are still poorly understood. We assessed edge effects at contrasting spatial scales across Europe and quantified the extent to which edges can preserve the seeds of forest specialist plants in temperate European deciduous forests along a 2,300-km latitudinal gradient. Through a greenhouse germination experiment, we studied how edge effects alter the density, diversity, composition and functionality of forest soil seed banks in 90 plots along different latitudes, elevations and forest management types. We also assessed which environmental conditions drive the seed bank responses at the forest edge versus interior and looked at the relationship between the seed bank and the herb layer species richness. Overall, 10,108 seedlings of 250 species emerged from the soil seed bank. Seed density and species richness of generalists (species not only associated with forests) were higher at edges compared to interiors, with a negative influence of C: N ratio and litter quality. Conversely, forest specialist species richness did not decline from the interior to the edge. Also, edges were compositionally, but not functionally, different from interiors. The correlation between the seed bank and the herb layer species richness was positive and affected by microclimate. Our results underpin how edge effects shape species diversity and composition of soil seed banks in ancient forests, especially increasing the proportion of generalist species and thus potentially favouring a shift in community composition. However, the presence of many forest specialists suggests that soil seed banks still play a key role in understorey species persistence and could support the resilience of our fragmented forests.

# POSTERS

## 1.1 = UAV-based hyperspectral monitoring of the microalgal community in a brackish environment: Lago delle Nazioni (Ferrara, Italy) case study

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Transitional and brackish waters span the freshwater-to-marine continuum and are considered among the most vulnerable coastal systems due to both environmental and anthropogenic stressors. In these areas, river runoff, as well as industrial and agricultural effluents, contribute significantly to the variability. Primary production and phytoplankton dynamics and distribution are closely related to environmental fluctuations of several factors such as temperature, light, salinity, water column stratification, grazer's pressure, nutrient availability; therefore, microalgae can also be used for water quality monitoring. Additionally, phytoplankton may directly impact water quality modifying water colour, dissolved oxygen concentration, turbidity, nutrients availability, thereby affecting different ecosystem processes at the very bottom of the environmental and trophic scale. Furthermore, phytoplankton cells proliferation could lead to production of toxins that may affects human health and other living-organisms. Understanding the mechanisms driving phytoplankton dynamics is crucial for predicting the impact of environmental changes, especially in threatened ecosystems. Monitoring activities are of great importance for controlling and managing algal communities and blooms that may occur. Traditional phytoplankton monitoring methods rely on microscopy which is well known to be extremely time-consuming and rely on the operator's skill level. Remote sensing offers a simple, accurate, and rapid detection technique for monitoring microalgae in the field and could be utilized as an ecological early warning system for detecting blooms. Specifically, hyperspectral remote sensing has great potential because it may allow the detection of different microalgal groups due to their peculiar pigmentary sets. This study aims to develop an innovative hyperspectral application to monitor phytoplankton in brackish environments including laboratory calibration, using monospecific microalgal cultures, and data field validation, using an unmanned aerial vehicle (UAV) to collect hyperspectral data on a coastal brackish water lake (Lago delle Nazioni) in the province of Ferrara (Italy). Despite the rapid growth of studies based on UAV systems, the number of applications in the field of microalgae monitoring is still limited. Abundances data were used to identify taxonomic groups that were more relevant, specifically Chlorophyta, Bacillariophyta and Miozoa. Spectral outcomes have enabled the detection of different absorbance and reflectance peaks for each algal species allowing to discriminate them. Moreover, good correlation between microalgal concentrations (cells density and chlorophyll-a) and spectral features were obtained during laboratory trials. Starting from laboratory tests, several spectral indexes were developed using different band ratios to identify main features of each species. Results showed good correlation between algal blooms specific indexes and phytoplankton biomass. Finally, examining reflectance of in-field remote sensed data, spectral signatures overlap with those obtained during calibration trials indicating how species-specific features could be observed even in environmental community conditions. In this study, we demonstrate how UAV hyperspectral data can be effectively used to monitor microalgae. These findings could be implemented by further analyses to separate and quantify different algal taxonomic group in environmental conditions. Our results provide a preliminary basis for setting new and integrated phytoplankton monitoring plans and scaling up the methodology to satellite applications.

## 1.2 = Tracking the origin of arbuscular mycorrhizas in *Anthoceros agrestis*

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Arbuscular mycorrhizal (AM)-like symbiosis appeared over 400 million years ago and are thought to have played a major role in plant transition from aquatic to terrestrial environments. During evolution, the genetic programme for AM has been recruited by other plant root symbioses such as legume symbiotic nitrogen fixation.
In angiosperms, AM development relies on fungal recognition through the perception of water soluble myc-factors that trigger symbiotic responses in the host plant. These include calcium-mediated signal transduction and plant cell reorganization. Hornworts, as the earliest diverging lineage in bryophytes, are excellent experimental systems to infer character evolution in the most recent common ancestor (MRCA) of land plants. Most hornworts interact with AM fungi and genome sequencing has revealed the presence of orthologues for symbiotic signaling genes in *A. agrestis*. Furthermore, a genetic trasformation protocol for this hornwort was recently published (1).
Here we present our recent results in the expression of fluorescent constructs in *Anthoceros agrestis* Paton to investigate early symbiotic responses. In particular, we investigate calcium-mediated signaling using confocal live imaging of a nuclear targeted R-GECO (red genetically-encoded calcium indicator). In addition, we take advantage of a mVenus tag for the endoplasmic reticulum to highlight host cell reorganization upon fungal contact and penetration. To this aim, we also developed a method for in vitro mycorrhization of *A. Agrestis*.

1) E. Frangedakis, M. Waller, T. Nishiyama, H. Tsukaya, H., X. Xu, Y. Yue, M. Tjahjadi, A. Gunadi, J. Van Eck, F.-W. Li, P. Szövényi, K. Sakakibara (2021), New Phytologist 232, 1488-1505. https://doi.org/10.1111/nph.17524

## 1.3 = Ultrastructural features of the grana stacks in wheat lines with different chlorophyll content

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In the chloroplast of land plants, the thylakoid system is defined by two morphological domains, appressed grana stacks and unstacked stroma lamellae. This unique organization results from the photosynthetic complexes segregation between thylakoid domains. Specifically, photosystem I (PSI) and ATP synthase are found in the stroma-exposed membranes, while photosystem II (PSII) is enriched in the grana core. The bipartite thylakoid architecture and its dynamism play an important role in the function and light acclimation of the photosynthetic apparatus in a changing environment. The level of granum irregularity can be an ultrastructural trait influencing photosynthetic flexibility.
Our study focuses on the variations of the grana organization occurring in outdoors-grown wheat mutants characterized by low chlorophyll content and defective control of photosynthetic electron transport. *Triticum aestivum* ANK-32A and *Triticum durum* ANDW-7B were compared to their corresponding WT lines, NS67 and LD222, respectively. TEM images of chloroplasts were used to calculate grana ultrastructural parameters. Photosynthetic parameters were obtained by modulated chlorophyll fluorescence and applying Light Curves (LC) and Rapid Light Curves (RLC) protocols. For each photosynthetic parameter the difference Δ(RLC – LC) was calculated to assess differences in the flexible response to light in the examined lines.
Our results indicate that the genetic defect of wheat mutants affected the thylakoid organization with different severity depending on the mutant. The reduction of the thylakoid system in ANK-32A was accompanied by a lower granum stacking degree. In ANDW-7B, only sparse grana were observed and the grana-intergrana organization was largely replaced by thylakoid overlaps and single thylakoids. A comprehensive analysis of the four lines indicated that the number of thylakoids per granum correlated positively with the total chlorophyll content. Both parameters correlated positively with the maximum non-photochemical quenching developed during the LC ($NPQ_{max}$), and negatively with the capacity to control the electron flow, as assessed by the yield of the non-regulatory energy dissipation ΔY(NO). Very interesting was the positive correlation between the chlorophyll *a*/*b* ratio and the Granum Lateral Irregularity (GLI), indicating that the PSII antenna size influences the thylakoid shifting in the lateral plane. However, no significant correlations were found between granum regularity indexes and functional parameters.

## 1.3 = Elemental composition changes in leaves of wheat lines differing in chlorophyll content

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Wheat mutants characterized by a lower chlorophyll synthesis than the corresponding wild-type lines have been considered instructive plant materials with respect to many aspects related to the structure and function of the chloroplast. However, a defective chlorophyll synthesis can also cause other non-obvious side effects on the plant. In particular, it is well known that the chlorophyll synthesis and the assembly of the photosynthetic machinery requires a set of metal ions, as micronutrients absorbed from the soil. Therefore, a partially impaired chlorophyll synthesis due to genetic mutations could result in an imbalanced elemental composition of leaves; if this is the case, the severity of the elemental composition disorder should correlate with the severity of the photosynthetic phenotype. To test these hypotheses, eight lines of either bread wheat (*Triticum aestivum*) or durum wheat (*Triticum durum*), which differ in their chlorophyll accumulation, were analyzed with respect to their elemental composition in comparison with the geochemical composition of the soil.
Grains were sown during the autumn in a parcel of the Botanical Garden of Ferrara. During the spring, after the tillering phase, the leaves were characterized in terms of photosynthetic parameters (chlorophyll content, chlorophyll *a* fluorescence, electrochromic shift) and then were sampled, together with the soil, for the geochemical analyses. The major element composition of the soil was characterized by X-ray Fluorescence (XRF); high-throughput inductively-coupled triple-quadrupole plasma-mass spectrometry (QQQ-ICP-MS) was used to determine the element concentrations down to the ultratrace in leaves and soil.
The mutants presented a complex photosynthetic phenotype; to reduce complexity, a principal component analysis of the photosynthetic parameters was run and the principal component explaining 70% of the variability (PC1) was used to the scope of the subsequent correlative analyses with the elements. In both wheat species, some negative correlations were found between PC1 and Mg, Al, P. Very interesting and unexpected was the relationship between PC1 and the rare earth elements (REEs). The relative abundance of REEs in the wild-type lines closely followed the trends in the soil but was characterized by a selective enrichment in Europium (Eu) and depletion in Thulium (Tm). In the leaves of the mutants, an increased REEs concentration was found; the heavy REEs especially, which were present in the leaf tissues as ultratraces, appeared very strongly correlated to the phenotype severity and more characterizing than the major elements. The selectivity for Eu or Tm uptake was lost. Collectively, the results indicate that leaf elementomics can contribute significantly to the phenotypic characterization of chlorophyll-deficient mutants and opens up a new application of REE chemometrics in plant biology.

## 1.3 = Cellular and electrophysiological investigations of extrafloral nectaries in *Vicia faba* during the plant-ant mutualistic relationship

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Extrafloral nectaries (EFNs) are specialized secretory structures that have been identified in over one hundred plant families worldwide. EFNs produce a carbohydrate-rich nectar that attracts arthropods, especially ants, which in turn offer protection to the plants. This mutualistic relationship has been studied for a long time. Previous studies (1,2) have shown how mechanical stimulation of the nectaries by ants induces the production of extrafloral nectar, thereby supporting the notion that EFNs are structures involved in plants' defense. The study of ant-plant mutualism has been a topic of interest for ecologists and evolutionary biologists for decades. While the role of extrafloral nectar (EFN) in this mutualistic relationship has been extensively studied, there is still much to be learned about the physiological characteristics of EFNs and their effects on the secreting cells, known as trichomes. The identification of EFN's physiological traits and a more thorough morphological studies will contribute to a better understanding of these structures and their role in the relationship with ants.
In this perspective, we used as a model organism *Vicia faba*, which has EFNs, and we adopted a multidisciplinary approach associating microscopic (optical microscopy, confocal microscopy, TEM) and electrophysiological analysis. Stimulating *Vicia faba*`s EFNs by ant bite (*Crematogaster scutellaris*), using microscopy, we found relevant morphological changes at the trichomes' ultrastructure level. We also identified substantial changes in the accumulation of secondary metabolites such as terpenes. Additionally, microelectrodes and Multielectrode Array Technology (MEA) were utilized to record and analyze a specific electrical response on EFN to ant bites: a characteristic depolarization of the electrical signal. Using different and specific channel inhibitors, we were able to have indications on the nature of the electrical signal. The data obtained from electrophysiological studies indicate that EFNs are electrogenic structures capable of generating an electrical signal in response to a mechanical stimulus. This signal, in turn, causes an internal physiological response to the trichomes as well as the modification of specific metabolic pathways.

1) Radhika, V., Kost, C., Mithöfer, A., & Boland, W. (2010) Proceedings of the National Academy of Sciences, 107(40), 17228-17233.
2) Bixenmann, R. J., Coley, P. D., & Kursar, T. A. (2011) Oecologia, 165, 417-425.

## 1.3 = Exploring the role of Dimethyl sulfoxide in *Posidonia oceanica*, a seagrass producing large amount of this molecule

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Dimethyl sulphide (DMS), Dimethyl sulfoniopropionate (DMSP) and Dimethyl sulfoxide (DMSO) are interrelated compounds that constitute an integral part of the marine sulfur cycle and play an important role in the global sulfur budget (1). Their biogenic production is, however, taxon-dependent and large producers are confined to a few classes of micro- and macroalgae. Unlike algae, observations of DMSP (and DMSO) in higher plants are rare, Borges and Champenois (2), demonstrated the occurrence of DMSO in the seagrass *P. oceanica.* The DMS was further reported to be the main volatile organic compound (59.3%) in *P. oceanica*. From this evidence, *P. oceanica* resulted as a top producer of DMSP and DMSO among marine and intertidal autotrophs, with foliar contents reaching up to 265 μmol. $gfw^{-1}$ for DMSP and 13 μmol. $gfw^{-1}$ for DMSO (Richir et al., 2020). The production dynamics of the two molecules in *P. oceanica* are closely linked and depend more on the plant's annual growth cycle than on environmental variables. Inter-annual variations in DMSO content in relation to shallow water temperature might further indicate that DMSO could have been involved in the physiological response of *P. oceanica* to heat stress. Scarce information is currently available on biosynthetic pathways of these molecules in seagrasses, the physiological functions they play and eventually, the evolutionary reasons for such a high production in *P. oceanica*.

The present work aims at determining the effects of dimethyl sulfoxide (DMSO) deliberate treatment on the seagrass *Posidonia oceanica* shoots acclimated in mesocosm. Seagrass shoots were collected by SCUBA diving at the Cirella island beds, Calabria. Shoots were acclimated in two mesocosms under controlled conditions of light regime, pH, and temperature. After seven days of acclimation, shoots were divided in five sub-cultures and each treated with 0.01%, 0.1%, 1%, 10% and 100% of DMSO in sea water; a further sub-culture was treated with pure sea water as control test. At weekly interval, leaf elongation, new leaves formation, leaf mortality and chlorophyll content were measured; proteomics analysis were performed at each sampling times. Leaf phenology and biochemistry suggested that DMSO positively affected the growth, reduced mortality, and enhanced the chlorophyll content in a dose-response manner with best effects at the 1% DMSO and worst effects at the 100% DMSO. On the side of molecular analyses, results of Differentially accumulated proteins (DAPs) among DMSO at various concentration in respect to control leaves, Gene Ontology (GO) and functional processes whose DAPs are belong will be presented; the KEGG pathway enrichment analysis allows to understand which biosynthetic pathways are significantly affected by external DMSO treatments. Finally, correlation among morphological, biochemical, and molecular results will be discussed.

1) E. Asher, J.W. Dacey, D. Ianson, A. Peña, P.D. Tortell (2017) J. Geophys. Res. Ocean., 122, 3269–3286.
2) A.V. Borges, W. Champenois (2017) Aquat. Bot., 143, 8–10.
3) J. Richir, W. Champenois, G. Engels, A. Abadie, S. Gobert, G. Lepoint, J. Silva, R. Santos, D. Sirjacobs, A.V. Borges (2020) Front. Ecol. Evol., 7, 510.

## 1.3 = Vibration perception in plant

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As sessile organisms, plants have developed alternative strategies compared to the animal kingdom to perceive the environment. While for the perception of light, temperature, and pathogens, multiple studies are ongoing, much less investigated is the perception of sounds in terms of mechanical vibrations. Evidence demonstrates that plants are indeed capable to respond to mechanical stimulation that is for instance induced by the pollinators` wingbeat. Multiple are the plant morphological factors that might contribute to the perception of vibration. In addition to the most evident structures such as the trichomes, there are intercellular and intracellular factors such as the mechanoreceptors and the membrane system in which they are integrated. We take advantage of a multidisciplinary team that was put together for the DAMATIRA project (aDvanced Analysis and Modeling of AcousTIc Responses of plAnts) with the aim to investigate plant-animal interaction in coevolutionally and ecologically relevant conditions dependent on vibration.
Specific sounds produced by insects during movement and feeding have been recorded. Using a system that reproduces the recorded frequencies and exploiting mutants defective for the trichome formation, we analyzed the changes in stress response, Reactive Oxygen Species (ROS) Accumulation and cell membrane potentials.
This investigation will contribute to decouple the effect of trichomes from the perception of membrane anchored mechanoreceptors and help to establish the stress response pathway that are specifically activated under the examined conditions.

## 1.4 = Screening of plant growth promoting fungi in a perspective of restore degraded areas and increase ecological functions

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The seed is an important stage in the plant life cycle with respect to its survival as a species, which is also ensured by its capacity to going into dormancy (1). Using this strategy, seeds overcome periods of unfavourable conditions and they ensure the successful of germination during a more favourable period. Dormancy is controlled by several environmental factors, such as light, temperature, and mechanical or chemical scarification. Seeds in the soil interact with microorganisms that could help to break dormancy. As the hyphae elongate, the fungi can crack the endocarp, potentially reducing the mechanical resistance to germination (2). Moreover, plant growth promoting fungi (PGPF) are known to stimulate plant thanks to their capacity to solubilize phosphate, make nutrients more readily available, suppress disease and control stresses (3).
The aim of this work was to evaluate screening tests for fungal strains useful for seed scarification and plant growth promotion. For this purpose, dilutions of soil were distributed on the following three different selective media. Blue Agar CAS medium was used to evaluate fungal production of siderophores, low-molecular-weight iron-chelating compounds, which allowed to solubilize $Fe^{3+}$ promoting its uptake. Pikovskaya's agar medium was used for detection of phosphate solubilizing soil fungi, in order to isolate fungal strains useful to promote phosphate assimilation in nutrient-poor soil. Moreover, colorimetric laccases tests were performed to screen fungi potentially involved in seeds scarification. These methods allow to select fungal strains that may be fundamental for the restoration of damaged areas, becoming tools for implementation of Nature Based Solutions. Furthermore, increasing absorption of nutrients and minerals by plants, fungi participate in improving ecosystem functions. This makes it possible, on the one hand, to increase the biodiversity of an area and, on the other hand, to regenerate and restore areas that have been degraded by natural disasters or anthropogenic pressure.

1), B.C.P. Samarasinghe, K.G. Jayasuriya, A.T.A. Gunaratne, M.C. Senanayaka, K.W. Dixon (2022). Seed Science Research, 32(2), 94-103.
2) P. Delgado-Sánchez, M.A. Ortega-Amaro, J.F. Jiménez-Bremont, J. Flores (2011). Plant Biology, 13(1), 154-159.
3) M.M. Hossain, F. Sultana (2020). Organic Agriculture, 1-31.

## 1.6 = Poplar response to salt stress using CRISPR/Cas9 technology

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Poplar (*Populus*) is an important forest tree that belongs to the *Salicaceae* family and comprises 29 diverse species, distributed in temperate, sub-temperate, and sub-tropical areas of the Northern Hemisphere (1). It grows fast and can adapt to survive in different environmental conditions. Additionally, poplar trees give humans access to raw materials for the paper industry, fuel wood, and lumber. More recently, they are being used as a potential biofuel source (2). It is considered a model tree for woody plants for molecular and genetic research because of its ease of cloning and availability of protocols for *in vitro* cultivation; it is also the first tree species whose genome has been completely sequenced (1, 3). Now the researchers are more interested in studying the effect and response of poplar trees to abiotic stresses such as salinity, which is considered as the most challenging abiotic stress that causes damage to plant growth and development.
Our study is based on the generation of *Populus alba* L. "Villafranca" clone *knock-out* lines of several genes using CRISPR/Cas9 technology to study their molecular characteristics and their gene network in response to salt stress. The *NAC13* (NAM (no apical meristem, Petunia), ATAF1–2 (*Arabidopsis thaliana* activating factor), and CUC2 (cup-shaped cotyledon, *Arabidopsis*)) transcription factor, a gene that participates in plant growth and response to external stress. Na+/H+ antiporter 1 (*NHX1),* a gene that is involved in the regulation of a wide variety of physiological processes such as trafficking, pH regulation, K+ homeostasis, protein transport, and plant development; and high-affinity K+ transporter 1 (*HKT1),* a gene that is involved in salt tolerance by contributing to Na+ exclusion from roots and shoots.
In the literature, the morphological and physiological characterization of the overexpression lines of the three genes showed enhancement in salt tolerance by improving the efficiency of antioxidant systems compared to the wild type under salt stress conditions. Our study focused on the generation of *Populus alba* L. "Villafranca" clone *knock-out* mutant lines using CRISPR/Cas9 technology to study their molecular, morphological, and physiological characteristics. Consequently, a bioinformatics analysis was carried out to check the copy numbers of the three genes using public databases (NCBI, Phytozome), and then a set of gene-specific primers were designed using bioinformatics tools (NCBI, Primer3plus, and Thermo Fisher) on conserved sequences in order to amplify the full length of the genes of interest. The genomic DNA was extracted from *in vitro* plantlets (Fig. 1), and the cloning was done by inserting the purified PCR product(s) of three genes into a suitable cloning vector and then transforming a specific *Escherichia coli* strain. Our next steps will be the sequencing of the positive colonies, the design of gRNAs, and *Agrobacterium tumefaciens*-mediated transformation.

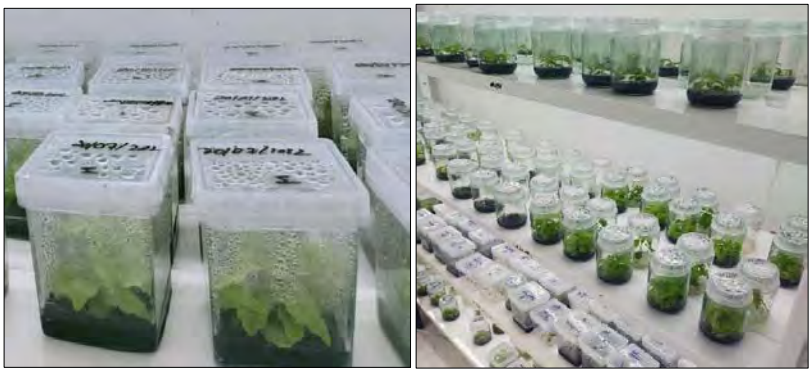

Fig 1. *In vitro* culture of *Populus alba* L. 'Villafranca' clone.

1) G.A. Tuskan, S. Difazio, S. Jansson, J. Bohlmann, I. Grigoriev, U. Hellsten, D. Rokhsar (2006). Science, 313(5793), 1596-1604.
2) A.K. Thakur, P. Kumar, N. Parmar, R.K. Shandil, G. Aggarwal, A. Gaur, D.K. Srivastava (2021) Forests, 52, 889-920.
3) A.M. Brunner, V.B. Busov, S.H. Strauss (2004) Trends in Plant Science, 9(1), 49-56.

## 1.6 = The Effect of PBAT Micro-Bioplastic on Lettuce Seed Germination (*Lactuca sativa*)

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Bioplastics have been proposed as more sustainable alternatives to conventional petroleum-based plastics (Venâncio et al., 2022). Conventional plastic mulches are designed to suppress weeds, retain moisture, and alter soil temperature in agriculture, while bioplastic mulches are supposed to do the same (Rajgadia and Debnath, 2022). PBAT is certainly one of the materials with the most commercial interest among bioplastics (Aversa et al., 2022), which can be used as a mulch film. The aim of this study is to investigate the potential phytotoxicity of PBAT during seed germination as the first growth stage. PBAT micro-bioplastic (size: 150-300 μm) was homogeneously suspended in water for 2 hours, and different concentrations were prepared. Then, 2 ml of the desired concentration of the suspensions were placed in 10-cm Petri dishes with 2 paper filters. Seeds of lettuce (*Lactuca sativa* L., Var. "Romana") were soaked for 2 hours and 20 seeds were sown in each treatment with 3 replicates for 72 hours. The number of germinated seeds and their length were measured every 24 hours for 3 days. The treatments of the experiments included control, 100 $mg.L^{-1}$, 500 $mg.L^{-1}$ and 1000 $mg.L^{-1}$ micro-PBAT. Statistical analysis of germination index (GI), germination percentage and relative GI was also performed using LSD test and independent t-test with R software. The data obtained showed that seed germination was significantly delayed in the first 24 hours of the experiment. From day to day, the delay decreased, and after 72 hours, there was no difference. Results from GI also showed the same trend. Thus, the presence of PBAT micro bioplastic has no significant phytotoxicity on the germination of lettuce seeds. The results are promising evidence for the safety of PBAT application in agriculture, but further studies are needed to ensure the safe application of PBAT in agriculture and to know the interactions between plants and bioplastics.

1) C. Aversa, M. Barletta, G. Cappiello, A. Gisario (2022) European Polymer Journal, Volume 173, 111304, https://doi.org/10.1016/j.eurpolymj.2022.111304.
2) N. Rajgadia, M. Debnath (2022) Biodegradable mulch utilizing bioplastic biopolymer polyhydroxyalkanoates, Materials Today: Proceedings, ISSN 2214-7853, https://doi.org/10.1016/j.matpr.2022.12.167.
3) C. Venâncio, I. Lopes, M. Oliveira (2022) Chemosphere, 309(2), 136810, https://doi.org/10.1016/ j.chemosphere. 2022.136810.

## 1.6 = Study of phenotypic analysis, cellular components organization and stress molecular pathways upon manganese exposure in *Arabidopsis thaliana*

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Manganese (Mn) is an essential element for plant growth and development. Mn is an essential cofactor for the oxygen-evolving complex (OEC) in photosystem II (PSII) (1), detoxification of reactive oxygen species (ROS), isoprenoid synthesis, and abscisic acid (ABA) and auxin signaling. Despite the fact that manganese deficiency may impact the aforementioned metabolic processes, excessive amounts of this metal are toxic to plants. Chlorotic leaves and necrotic patches are the most prevalent indicators of manganese toxicity (2). The high concentration of manganese in the soil may be attributable to several processes; however, the anthropogenic release of waste into the soil and the use of intensive fertilizers are the most significant contributors. This is not simply a plant issue; the introduction of manganese into the food chain might have severe repercussions on human health. Some studies indicate that manganese can enhance the development of neurodegenerative diseases such as Alzheimer's disease or neuromotor disorders comparable to Parkinson's (3). Despite the burden that high levels of Mn have on plant physiology, our understanding of the systems that mediate Mn absorption, transport, and subcellular compartmentation is limited. This is mostly owing to a lack of knowledge regarding the expression and subcellular localization of manganese transporters in the majority of plant species, particularly at hazardous doses.
This study analyzes the harmful effects of manganese on the development and growth of *Arabidopsis thaliana*. Through phenotypic research, we wish to determine how different Mn concentrations influence the development of these plants' major organs. Using confocal microscopy, we determine how dangerous levels of Mn affect organelles and cellular structures, including mitochondria, chloroplasts, the Golgi apparatus, endoplasmic reticulum, the vacuole, and the cytoskeleton. We are also interested in determining how Mn-toxic circumstances alter cytosolic and endoplasmic reticulum protein homeostasis, which is essential for maintaining normal cellular function. By explaining the impacts of manganese toxicity on plants, this work will indirectly contribute to the understanding of the possible problems posed by excessive manganese levels in the environment and the necessity of moderating its accumulation. This research has the potential to increase our understanding of manganese toxicity in plants, elucidate cellular responses to stress, and contribute to human health concerns.

1) R. Millaleo, M. Reyes-Dìaz, A.G. Ivanov, M.L. Mora, M. Alberdi (2010) J. Soil Sci. Plant Nutr, 10(4), 470–481.
2) J. Li et al. (2019) International Journal of Molecular Science vol. 20.
3) A.C. Martins et al. (2019) Int J environ Res Public Health 16.

## 1.6 = Effects of Cadmium treatment on endoplasmic reticulum quality control in *Arabidopsis thaliana*: the key role of Glucosidase II enzyme in metal response

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Heavy metals are naturally present in the Earth's crust, industrialization causes their accumulation in all biological systems and their toxicity has become a global problem. The uptake, accumulation and detoxification mechanisms evolved by plants to survive high concentration of cadmium ions ($Cd^{++}$) are well documented in the literature, but little attention has been paid to the effects of heavy metals on Endoplasmic Reticulum (ER) stress. In a recent study we discovered that attenuation of the Unfolded Protein Response (UPR) pathway impairs sensing of ER stress and mediates $Cd^{++}$ tolerance in *Arabidopsis thaliana* plants (1). UPR is strictly linked to the Endoplasmic Reticulum Quality Control (ERQC) system, which tightly controls the correct folding and maturation of nascent glycoproteins so that only the correct folded ones proceed along the secretory pathway and reach their final cellular or extracellular destination. The ER alpha-glucosidase II (α-GII) is the ERQC gatekeeper, admitting folding glycoprotein clients into ERQC and releasing them from it (2).
We describe here for the first time the effects of chronic Cd++ treatment on the A. thaliana α-GII rsw3 mutant plant, which carries the S599F missense mutation near the catalytic site (3). We compare the phenotypic, biochemical and molecular responses of Wt and rsw3 plant lines to Cd++ treatment, and detect and report an increase tolerance of the mutant to stress conditions. The expression levels of the ER chaperone BiP3 are not increased in presence of Cd++, contrary to Wt plants, suggesting increased tolerance to ER stress in the mutant. The rsw3 mutant also exhibits defects in auxin signaling. These links between Cd++, ERQC and auxin signaling will be further investigated towards identification of novel genetic traits involved in toxic metal ion tolerance in plants.

1) M. De Benedictis, A. Gallo, D. Migoni, P. Papadia, P. Roversi, A. Santino (2023) Plant Physiol. Biochem, 196, 281–290.
2) R. Strasser (2018) Annu. Rev. Plant Biol. 69, 147–172.
3) J.E. Burn, U.A. Hurley, R.J. Birch, T. Arioli, A. Cork, R.E. Williamson (2002) Plant J, 32, 949–960.

## 1.6 = Increasing drought tolerance of lettuce (*Lactuca sativa*) with wood distillate

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Drought is one of the abiotic stresses that most affect the physiological status of crop plants. Therefore, alternative solutions are needed to increase plant tolerance to drought. Wood distillate (WD, also known as pyroligneous acid or wood vinegar), a by-product of pyrolysis of woody biomass, is known to improve the growth and yield of several crop plants. The possible benefits of WD application in increasing the drought tolerance of crop plants has yet to be studied in detail. In this study, lettuce (*Lactuca sativa* L. var Ranger), which is the most cultivated leafy vegetable worldwide and is particularly sensitive to drought, was selected as a model crop species to investigate the potential of WD application on the growth and vitality of lettuce under different drought stress conditions. Plants were treated with either water (control, C) or 0.5% (v/v) WD once per week by soil fertigation under three different drought stress conditions (no stress – NS, soil field capacity = 70%; moderate stress – MS, soil field capacity = 50%; high stress – HS, soil field capacity = 30%). At the end of the experiment, fresh weight, chlorophyll content, and total soluble protein (TSP) content were measured as indicators of plant growth, while malondialdehyde (MDA) content, total phenolic content (TPC), and total flavonoid content (TFC) were measured as stress biomarkers. The results showed that increasing drought stress negatively affects plant growth, and that WD has the potential to provide important benefits. The application of WD significantly increased plant biomass (24% and 26% at MS and HS, respectively) and TSP (28% and 19% at MS and HS, respectively), while MDA, TPC and TFC significantly decreased (up to 19%). Overall, these results suggest that the application of WD has a positive effect on the tolerance of lettuce plants to drought by increasing growth and reducing the stress. The main outcome of this study is that differences in yield caused even by severe drought stress are no longer evident after WD treatment.

## 1.6 = Ionomic profile in tomato (*Solanum lycopersicum* 'Micro-Tom') under Nickel stress

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Nickel (Ni) is the 24$^{th}$ most abundant element in the Earth's crust comprising about 3% of the Earth's composition. It is widely distributed in the environment, being released through both natural and anthropogenic activities (1). Being a constituent of urease, it is considered an essential element for plants at low concentrations (0.05–10 mg/kg dry weight) but at high concentrations it becomes toxic for plants. It can cause a disruption of photosynthesis, induce indirect oxidative stress, reduce plant's yield and growth and alter cells membrane that leads to DNA damage, gene mutation and lipid peroxidation (2). It is present in the air, water, soil and biological materials (1). Ni may affect also the quality and safety of the plants and their products, being present as heavy metal in wastewater used for irrigation (3). According to the ministerial decree of 12 June 2003, the limit value of Ni concentration in wastewater is 0.2 mg/L.
An ionomic characterization of roots, stems and leaves of *Solanum lycopersicum* L. in the pre-fruit stage under Ni stress has been performed. Micro-Tom plants were cultivated in the hydroponic system under controlled environmental conditions for 15 days renovating the treatments one time after one week. Three treatments of Ni were applied with half-strength MS solution (0.1, 0.2 and 0.4 mg $L^{-1}$ of $NiCl_2$) and compared to control plants (half-strength MS solution). During the experiment, SPAD units were measured. Root, stem and leaf samples were collected after 15 days. A sample of 300 mg of dry (60 °C until reaching constant weight) plant material (n=5) was digested in 8 ml of 65% $HNO_3$, using the COOLPEX Smart Microwave Reaction System. The digested samples were then diluted with Milli-Q $H_2O$ to a final volume of 30 mL and were then analyzed. Elements (Ni, K, Ca, Mg, Na, Zn, Cu, Mn and Fe) concentration were determined using a Microwave Plasma-Atomic Emission Spectrometer (4210 MP-AES, Agilent Technologies).
Our results showed no differences in SPAD units during the 15 days among the treatments. A significant decrease in root fresh weight was measured under 0.4 mg $L^{-1}$ of $NiCl_2$ compared to the control. The application of Ni resulted in a significant increase in its concentration following the order roots>leaves>stem according to the three different Ni levels applied. Additionally, a significant increase in K concentration in roots and Mn concentration in stems and leaves was detected when exposed to 0.1 mg $L^{-1}$ $NiCl_2$.
Further analyses are in progress to better understand if the presence of Ni can alter the metabolomic and transcriptomic profile in tomato plants.

Fig. 1. Micro-Tom cultivated in hydroponic system.

Fig. 2. Agilent 4210 MP-AES.

1) M. Cempel, G.J.P.J.S. Nikel (2006) Polish Journal of Environmental Studies, 15(3).
2) C. Chen, D. Huang, J. Liu (2009) Clean–soil, air, water, 37(4-5), 304-313.
3) A. Malakar, D.D. Snow, C. Ray (2019) Water, 11(7), 1482.

## 1.6 = Role of *Salt Overly Sensitive* (SOS) genes in *Populus alba* 'Villafranca' under salt stress mutated with CRISPR/Cas9 approach

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As the first tree species to have the entire genome sequenced, Poplar (*Populus* sp.) is considered nowadays a model tree for genetics and biotechnology research. Its adaptability to different environmental conditions, in a context where the adverse effects of climate change and soil salinization are increasing, makes it a good candidate for genetic improvement studies. In this perspective, our study focuses on evaluating the salinity response of *P. alba* L. "Villafranca" knock-out mutants for salt-stress related genes.
The CRISPR/Cas system, a gene editing technology, was used in this study to produce knock-out mutants. This system uses the Cas9 protein and specific guide RNAs (gRNAs) to target and modify genes at specific sites (1). The *Salt Overly Sensitive* (SOS) genes under study can improve tolerance to different abiotic stresses in woody plants, and in particular to salinity (2). The *Phytoene Desaturase* (PDS), a gene involved in the biosynthetic pathway of carotenoids, is used as a reporter gene since its inactivation determines an albino phenotype (3). Bioinformatics characterization of SOS and PDS genes was made using public databases to select the specific target sequences and *in vitro* plantlets at the stage of 4-6 true leaf were grown for the transformation.
Two different copies of a SOS gene were selected as the candidate targets for knock-out, and the PDS gene was selected in a single copy form. Specific primers were designed to target the conserved regions of interest. *E. coli* (DH10B) carrying the pJET 1.2 vector was used to transform the genomic DNA as the first step of the cloning procedure. Positive colonies were selected (Fig 1), and total DNA was sequenced using the Sanger approach. From these first results the exact sequences of PDS and SOS genes in *P. alba* "Villafranca" were obtained (Fig 2).
The following step was the design of a dual-guide RNAs for each gene, designed on the genomic sequences. *A. tumefaciens* and the PBV[2CRISPR]-Neo/Kana-zCas9-AtU6-26 plasmid construct, a binary vector containing gRNA sequences for targeted genome editing, selectable markers, and a modified Cas9 protein, to use for the transformation of the plantlets.
The CRISPR-Cas9 mutated lines will be analyzed to check for the presence of specific mutations by extraction, purification, and sequencing of the target regions. Knock-out mutants will be studied under salinity, varying the duration and the magnitude of the stress, at morphological, physiological, and molecular level. Enhanced resistance to salinity is expected.

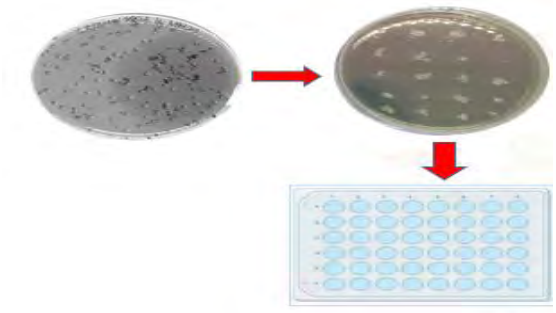

Fig. 1. Positive colony selection and sequencing preparation.

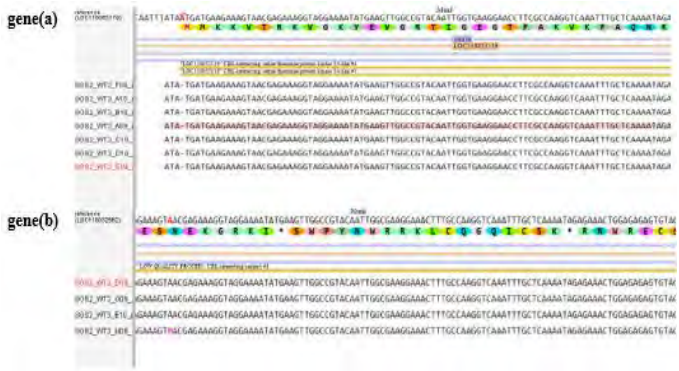


Fig. 2. Example of SOS2 sequences alignment with the reference gene (a) and gene (b).

1) K. Zhang, N. Raboanatahiry, B. Zhu, M. Li (2017) Frontiers in Plant Science, 8, 177.
2) S. Hussain, S. Hussain, B. Ali, X. Ren, X. Chen, Q. Li, M. Saqib, N. Ahmad (2021) Plant Physiology and Biochemistry, 160, 239-256.
3) S.W. Henderson, S.T. Henderson, M. Goetz, A.M. Koltunow (2020) Genes, 11(9), 1064.

## 1.6 = Novel approach to link the physiological response of maize to drought and the occurrence of mycotoxins

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Increased frequency of heatwaves and droughts are some of the aspects by which climate change threatens maize cultivation (*Zea mays* L.), thus playing a crucial role in global food security. The impact of drought on maize production is multifaceted. Firstly, drought stress during the critical stages of germination, flowering and pollination can lead to decreased plant growth, pollen production, nutrient uptake and ultimately to lower grain yields (1). In addition, prolonged periods of drought increase the spread of pests and diseases, which further exacerbate the vulnerability of maize crop and may compromise yield quality (2). However, the introduction of irrigation could only partially limit the damaging effects of severe water stress on maize performance. Therefore, improving the knowledge of the physiological response to water scarcity of this crop may be crucial for agronomic choices in the field.
In this context, we monitored 20 maize fields, characterized by the simultaneous presence of irrigated and non-irrigated areas, along a soil grain size gradient in the Friuli Venezia Giulia region throughout the growing season, at four phenological stages (*i.e.*, beginning of stem elongation, flowering, milk maturation, dent maturation). In particular, we considered the plant individual functional response (*i.e.,* plant height, SLA, leaf DMC, leaf chlorophyll, carotenoid and flavonoid content, kernel DW, kernel C:N, kernel $\delta^{13}C$), and the aflatoxin and fumonisin content of the kernel in relation to irrigation conditions and climate data (*i.e.,* total precipitation, mean temperature).
We found that the soil structure effect is overruled by the effect of climate and/or irrigation. As we expected the absence of irrigation and low total precipitation led to a reduction of plant biomass and kernel production whereas interaction between irrigation and phenological stage had significant effect on leaf pigments (*i.e.,* chlorophyll) and secondary metabolites (*i.e.,* flavonoid). Moreover, irrigation enhanced the kernel C:N ratio and $\delta^{13}C$, whereas reduced the amount of aflatoxins.
Our further investigations aim at evaluating the interplay between the climatic variables, the soil structure and the individual traits on the ultimate content of mycotoxins in the kernel, with two distinct irrigation regimes and at the given different phenological stages adopting a structural equation modelling approach.

1) A. Challinor, J. Watson, D. Lobell, S.M. Howden, D.R. Smith, N. Chhetri (2014) Nature Clim Change 4, 287–291.
2) S. Savary, L. Willocquet, S.J. Pethybridge, P. Esker, N. McRoberts , A. Nelson (2019) Nat Ecol Evol 3, 430–439.

## 2.3 = Production of fungal siderophores and organic acids for biomining perspective

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The global economy needs an ever-increasing amount of metals, including REEs, to maintain current production standards and develop new technologies. At the same time, primary resources from which metallic elements can be extracted by conventional techniques are being rapidly depleted, and mining industries are forced to turn to matrices containing smaller amount of elements and thus hitherto discarded (1). Extracting these elements by conventional techniques from waste minerals with low metal concentrations is difficult and polluting. For this reason, there is a strong need to develop new mining approaches that can implement extraction even on rocks with lower amounts of elements and, at the same time, decrease the environmental impact of these processes (2). Among these new extraction techniques, those based on microorganisms seem the most promising and are therefore increasingly being studied.
Fungi, that are heterotrophic organisms, may be used in biomining; this process is based on the organic acids they produce and can be applied to any ore containing metals in the form of oxides. In addition to the acid leaching process facilitated by acidogenic fungi, siderophores produced by selected fungal microorganisms are also involved in the mobilization and sequestration of metals. Siderophores are object of scientific investigation due to their capacity to solubilize metal ions and maintain it into chelate complex. Through the complexation of metals, the bioleaching of minerals could be facilitated, potentially enhancing their dissolution. Consequently, the supplementary metal sequestration capabilities exhibited by siderophores could potentially play a role in the bioweathering process of ores (3).
A series of experiments was conducted in our laboratory to select appropriate fungal microorganism strains for biomining purposes. These experiments aimed to assess the capabilities of different strains in performing biomining. Specifically, the tests were based on the premise that fungal biomining relies on the strain's capacity to produce organic acids, which play a fundamental role in the biomining process. Additionally, the potential of the strains to produce siderophores, molecules capable of mobilizing metal ions, and their tolerance to environments with high nutrient content and significant metal pollution were evaluated.
The tests were carried out on the strains belonging to the mycology laboratory collection. These strains were obtained from water samples seeping from rocks in the mountainous territory of the Fanes gorge (Cortina).
The tests were conducted sequentially, starting with the assessment of the strains' ability to produce siderophores on a specific medium using CAS tests. Subsequently, for strains that showed positive results, acid production and tolerance to heavily metal-contaminated media were evaluated. The acid production test involved inoculating the strains in a suitable medium and measuring the pH decrease induced by fungal growth. The tolerance test to metal-contaminated media was measured by comparing the radial growth of strains on both standard Sabouraud agar (SAB) and SAB agar supplemented with metals (SAB_M). The metal concentration in the SAB_M matched the average levels found in the percolating water from metalliferous mines in Tuscany.
This work allowed us to evaluate the potential of strains for their ability to perform biomining and tolerate metal concentrations suitable for this process. Furthermore, it indicates their potential for the detoxification (by metal removal) of solid and liquid substrates. Future research, prior to conducting actual biomining tests, will focus on assessing the growth potential of these strains in consortia to enhance their extraction capabilities.

1) I. Watson (2015) Mining and the Environment Introduction.
2) C.L. Brierley, J.A. Brierley (2013) Applied Microbiology and Biotechnology, 97(17), 7543-7552.
3) J. Kirtzel, N. Ueberschaar, T. Deckert-Gaudig, K. Krause, V. Deckert, G.M. Gadd, E. Kothe (2020) Environmental Microbiology, 22(4), 1535-1546.

## 2.5 = An update on the phylogeny of *Cecropia* (Urticaceae), with particular reference to the mirmecophytic species

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*Cecropia* Loefl. is an important neotropical genus, whose species represent important components of tropical forests from Mexico to Argentina (1). Samples of several species of *Cecropia* with particular reference to *C. obtusifolia* Bertol. were collected in Panama and used to obtain ITS and trnF-trnL DNA sequences. This genus is remarkable also for its typical capability of establishing mutualistic relationships with ants belonging to genus *Azteca*, occurring in 46 of 61 species of *Cecropia* (2). The aim was to test the monophyly of the species of *Cecropia* strictly mirmecophytes with respect to other less specialized species. The results showed that *C. sciadophylla*, which is not a myrmecophyte, is outgroup to the rest of *Cecropia*. Apparently the myrmecophyte mutualism was lost more than one time during the phylogeny of the genus.

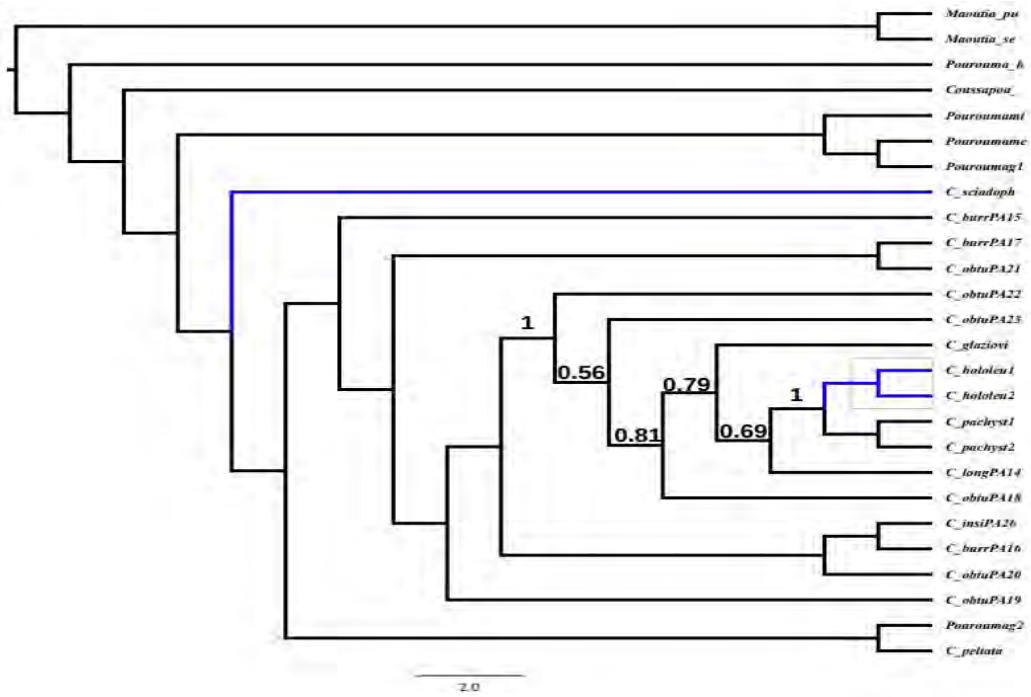


Fig. 1. Phylogeny of *Cecropia* and allied genera.

1) J.D.O. Santos, F.L. Zchonski, L. Pilati, A.L. Gaglioti, S. Romaniuc-Neto, P.R. Da-Silva (2020) Tree Genetics & Genomes, 16, 1-13.
2) J. Gutiérrez-Valencia, G. Chomicki, S.S. Renner (2017) Molecular Phylogenetics and Evolution, 111, 196-205.

## 2.6 = A new campion of the Albanian serpentine flora: *Silene isabellae* (Caryophyllaceae)

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*Silene* L. is the largest genus in family Caryophyllacae Juss., including ca. 870 species of annual and perennial herbs, distributed in the northern hemisphere, south America, and south Africa (1, 2). The number of European *Silene* species is currently estimated in about 200 and a large part of these is found in the Mediterranean countries, where about the 40% of these are endemic (according to 1). In particular, the south-western countries of the Balkan peninsula are major centres of species richness. According to 2, a total of 140 taxa of *Silene* taxa (species and subspecies) is found in Greece, while the most recent checklist of the Albanian flora (3) lists as many as 52 native taxa (excluding erroneously reported and doubtful taxa).
In the last decade, we had the opportunity to make several plant collections during our field trips in Albania and especially in the vast serpentine outcrops in the internal parts of this country, which were the basis for contributions on the Albanian flora. Among these collections, some specimens of a perennial species of *Silene* found in the understorey of open *Pinus nigra* forests and in rocky grasslands at 1000-1600 m a.s.l. on the ultramafics of the Skënderbëut mountains of central Albania could not be identified based on the available literature and herbarium materials.
Subsequent investigations revealed this plant to be a new serpentine endemic species likely belonging to section *Elisanthae* (Fenzl ex Endl.) Ledeb., which was named *Silene isabellae* Selvi & Bianchi (Fig. 1). This species shows affinities with the widespread European species *S. noctiflora* L., but it is sharply distinct from the latter species in habit, stem and leaf pubescence, morphology and biology of the flowers, and length of the carpophore. Moreover, the ecology of the two taxa is also contrasting, being *S. noctiflora* annual and synanthropic-ruderal, mostly occurring in lowland sites. Weaker similarities were also observed with the south European subalpine taxa of the group of *S. vallesia* L. of section *Auriculatae* (Boiss.) Schischk., though these are not likely to reflect a real systematic affinity.


Fig. 1. a) Distribution of *Silene isabellae.* b) Holotype of *S. isabellae.* c) d) Field photos of *S. isabellae.*

1) W. Greuter (1997) *Silene* L. In Strid A, Tan K (eds) Flora Hellenica 1, 239–323, Lubrecht & Cramer.
2) P. Dimopoulos, T. Raus, A. Strid (2018) "Flora of Greece" Web, version 2.
3) Z. Barina, G. Somogyi, D. Pifkó, M. Rakaj (2018) Phytotaxa, 378, 1–339.

## 2.6 = Disentangling the diversity of *Hieracium tenuiflorum* group with an integrated taxonomic approach

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The genus *Hieracium* s.str. is one of the most diverse and intricate vascular plant genera among the angiosperms. It is characterized by high morphological variation and the few phylogenetic studies conducted on this genus revealed complex pattern of reticulate relationships among taxa, which suggests that several hybridization events may have occurred in the past (1). The most complete taxonomic treatment of this genus was published by Zahn at the beginning of the last century, and the current taxonomic treatment in Italy (2) has not substantially changed Zahn's scheme. As a result of this, more than 1,300 taxa (species and subspecies) are reported in Italy (2).
Among them there is *H. tenuiflorum* Arv.-Touv., a species belonging to *H. murorum* L. group (section *Hieracium*), occurring in Alps and Apennines, where it grows in wood margins and along roadsides between 200-1800 m a.s.l. Currently, there are 6 accepted subspecies ascribed to *H. tenuiflorum: H. tenuiflorum* subsp. *austrosilvularum* (P.Rossi & Zahn) Gottschl.; *H. tenuiflorum* subsp. *glaucoviolascens* (Bornm. & Zahn) Gottschl.; *H. tenuiflorum* subsp. *pictoprasinum* (Fen. & Zahn) Gottschl.; *H. tenuiflorum* subsp. *pseudomerianum* (P.Rossi & Zahn) Gottschl.; *H. tenuiflorum* subsp. *sebini* (Fen. & Zahn) Gottschl.; *H. tenuiflorum* subsp. *tenuiflorum*(3). Most of these taxa were described for Italian localities across the Alps and few of them were no longer recorded.
In this work, we tried to disentangle the taxonomy and evolution of the *H. tenuiflorum* group using a multidisciplinary approach. We looked for original material identified by Zahn and stored in Italian and European herbaria (Bicknell's Herbarium; B; BERN; G; JE; FI; PAV; TR; W; Z), moreover we collected fresh material from *loci classici* of *H. tenuiflorum* subspecies and in different localities in the Alps and Apennines. We performed a genome size estimation and chromosome counts on living plants obtained by seeds germination, and a morphometric analysis on herbarium specimens and plant collected in the wild. As expected, preliminary results revealed indistinct morphological clusters between different subspecies, but we observed the presence of at least 3 diploid *H. tenuiflorum* populations. To the best of our knowledge this is the first observation of diploid taxa within the section *Hieracium* and provides new insights into the evolutionary history of the species in the Alps. Genetic analysis using three plastid markers and 10 nuclear microsatellite markers are ongoing, in order to investigate the phylogenetic history of this group and asses if there is gene flow between different subspecies. We expect that genetic information, jointly to our previous results, will lead to a better comprehension on *H. tenuiflorum* diversity and evolution.

1) P. Mraz, P. Zdvořák (2019) Ann. Bot., 123(2), 391-403.
2) F. Bartolucci, L. Peruzzi, G. Galasso, A. Albano, A.N.M.G. Alessandrini, N.M.G. Ardenghi, ... F. Conti (2018) Plant Biosyst., 152(2), 179-303.
3) G. Gottschlich (2013) Willdenowia 43(1), 157 (2013).

## 2.6 = Different species or altitudinal morphotype? Testing the taxonomic value of *Dianthus brachycalyx* (Caryophyllaceae)

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The sharp ecological gradient in mountain environments is an important driver of phenotypic differentiation of plants, which may result from speciation between high- and low-altitude populations or from the presence of intraspecific phenotypic variation (1). *Dianthus brachycalyx* A.Huet & É.Huet ex Bacch., Brullo, Casti & Giusso (Caryophyllaceae) is a small-sized carnation species, endemic to mountain peaks of the central-southern Apennines (2). The distribution range of this species is nested within that of *Dianthus virgineus* L., a widespread species mainly distributed across a broad altitudinal span from southern France to southern Italy (3, https://dryades.units.it/floritaly/index.php). Although *D. brachycalyx* should differ from *D. virgineus* for being smaller and having shorter leaves and calyx, the presence of morphological intermediates hampered a solid recognition of the two taxa. Here we present results of a systematic study aimed to unveil the relations between *D. brachycalyx* and *D. virgineus*, to test whether the former taxon is indeed a separated species or it represents just a high-altitude morphotype of the latter. We applied an integrated approach based on morphometry, genetics (ddRAD-seq), and climatic niche comparison. We sampled 25 French and Italian populations, including those from type localities of *D. brachycalyx* and *D. virgineus*. We collected herbarium specimens for morphometric data acquisition, and silica-dried leaves for DNA extraction. For niche reconstruction, we obtained occurrence data from online databases, herbaria, and personal observations. Without imposing *a priori* taxonomic assumptions, there is no clear recognition of the two species in the morphospace, but there are broad overlaps between *D. brachycalyx* and montane populations of *D. virgineus,* both showing a generally reduced size of leaf and flower features. Genetic variation is low, reflecting the geographic distribution of the studied populations rather than their taxonomic identity. Climatic niches of the two species are moderately overlapping and similarity tests were non-significant, suggesting that niches are different due to different availability of environmental conditions within each species range. Thus, our data support the hypothesis that *D. brachycalyx* and *D. virgineus* belong to a single species, expressing different altitude-related morphotypes with no taxonomic value.

Acknowledgements
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1) A.H. Halbritter, S. Fior, I. Keller, R. Billeter, P.J. Edwards, R. Holderegger, S. Karrenberg, A.R. Pluess, A. Widmer, J.M. Alexander (2018) J. Evol. Biol., 31, 784-800.
2) G. Bacchetta, S. Brullo, M. Casti, G.P. Giusso del Galdo (2010) Nord. J. Bot., 28, 137-173.
3) G. Domina, G. Astuti, G. Barone, D. Gargano, L. Minuto, L. Varaldo, L. Peruzzi (2021) Taxon, 70, 1096-1100.

## 2.6 = The geographic mosaic of biotic interactions drives local adaptation in the cliff species *Dianthus rupicola* (Caryophyllaceae)

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Spatial variation in selective pressures and strong natural barriers which limit gene flow among populations can drive the evolution of local adaptation within species. In plant populations, biotic interactions can impose consistent divergent selection by influencing the ecology of plant reproduction and the evolution of plant traits. Particularly, the combined action of mutualistic and antagonistic interactions plays a key role in promoting adaptive differentiation. Cliff habitats due to their high geographic fragmentation and their complex mosaic of biotic interactions thus represent an excellent system to investigate the mechanisms underlying local adaptation in plant species.
Here, we focused on *Dianthus rupicola*, a chasmophyte, strictly related to cliff habitat and endemic to central Mediterranean. This species represents a particularly useful model in local adaptation studies due to its highly fragmented distribution and its mutualistic (pollination) and antagonistic (floral herbivory) interactions which can impose different selective pressures on floral traits. To test our hypotheses, we analyzed six populations localized in peninsular southern Italy, Sicily and Aeolian islands and specifically addressed the following questions: (1) Is there phenotypic differentiation among *D. rupicola* populations? (2) If so, which role do biotic factors play in driving phenotypic divergence? (3) Do biotic factors affect plant reproductive success?
To examine phenotypic divergence among populations, in each population we randomly selected 20 individuals and measured plant traits potentially attractive for both pollinators and floral herbivores. First, for each sampled individual we recorded: (1) total number of flowers, (2) number of flowers per inflorescence, (3) number of open flowers per inflorescence. Then, we selected 3 fully-opened flowers per plant to account for variation within single individuals and collected (1) morphological data by scanning each flower through a 3D Lidar system and (2) flower colour. Moreover, we characterized the pollinator and floral herbivore community structure and at the end of flowering season on each individual we thus estimated plant reproductive success measured as number and dimension of seeds. To examine the relationship between mutualistic and antagonistic interactions, plant traits and reproductive success we used piecewise structural equation models (SEM). Separate models were built grouping together populations with similar biotic interactions.
Results showed significant differences among populations in floral traits found to be under selection and in reproductive success. Interestingly, these differences were explained by a complex mosaic of biotic interactions: populations were characterized by a different degree of pollination specialization and a highly different percentage of infection caused by floral herbivores or nursery pollinators. According to our findings phenotypic and reproductive success variation are thus the result of the combined effect of mutualists and antagonists, particularly, in highly infected populations plants possessing the combination of traits simultaneously enhancing pollination and escape from herbivores showed a significative fitness advantage.
Our study thus provides insights into the patterns and causes of variation in local adaptation of this cliff species, highlighting the importance of considering mutualistic and antagonistic interactions, beyond their additive effect, as drivers of adaptation in long-lived, pollinator-dependent plants.

## 2.6 = Genetic and morphological assessment of *Helichrysum* from the Tuscan Archipelago (Italy)

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In the Mediterranean area, the species of the genus *Helichrysum* Mill. (Asteraceae) present high genetic similarity despite they can have different morphological characteristics (1). In the Tyrrhenian area, wild *Helichrysum* plants are harvested as a medicinal source, for their antimicrobial and anti-inflammatory activities. Despite the numerous morphological/morphometric investigations carried out to clarify its taxonomic complexity (2), many doubts remain, taxonomic revisions are often discordant, and most populations included in the *H. italicum* complex still present numerous taxonomic uncertainties. In the Tuscan Archipelago, the most recent studies reported the occurrence of *Helichrysum italicum* (Roth) G. Don subsp. *italicum*, *H. litoreum* Guss. and *H. stoechas* (L.) Moench (Fig. 1) (3). In this study, 12 populations of *Helichrysum* from this Archipelago were analysed: 6 from Elba, 3 from Capraia and 3 from Giglio, including a total number of c. 180 individuals. For comparison, we also included c. 50 reference specimens belonging to the three *Helichrysum* taxa previously reported for the Tuscan Archipelago but from other geographic areas. ISSR analysis (Inter-Simple Sequence Repeats, 88 *loci* individuated by Capillary Electrophoresis analyser) and morphological assessments (27 traits, measured by a Digital Optical Microscope, 250× max magnitude) were carried out on all the specimens. As a result, both morphological and genetic analyses showed the most of variability at the intra-population level, as revealed by the coefficient of variation and AMOVA-Gst. The high degree of morphological and genetic similarity among the Tuscan Archipelago populations, and the lack of clear gaps in the pattern of variation, cannot allow to justify the identification of more than one species. Moreover, all twelve populations of the Tuscan Archipelago make a unique cluster with the *H. italicum* reference specimens.

Fig. 1. Herbarium samples of *Helichrysum* analysed (from left to right): *Helichrysum italicum* (Roth) G. Don subsp. *italicum*, *H. litoreum* Guss. and *H. stoechas* (L.) Moench subsp. *stoechas.*

1) S. Herrando-Moraira, P. Carnicero, J.M. Blanco-Moreno, L. Sáez, E. Véla, R. Vilatersana, M. Galbany-Casals (2017) Taxon, 66, 909–33.
2) M. Galbany-Casals, L. Sáez, C. Benedí (2006) Canadian Journal of Botany, 84,1203–32.
3) A. Carta, L. Forbicioni, G. Frangini, B. Pierini, L. Peruzzi (2018) Italian Botanist, 6, 1–22.

## 2.6 = Toward a better understanding of the *Festuca valesiaca* complex (Poaceae) in Italy

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*Festuca* L. is one of the most species rich genus of grasses (Poaceae) family, counting over 500 species. The highest species richness is concentrated in the type *Festuca* subgenus *Festuca*, commonly known as fine-leaved fescues. This group dominates various xerophytic and alpine communities over the whole Europe and are of a particular economical interest being used in restoration ecology. The xerophytic and cryophytic adaptation makes individual taxa very similar and these fine-leaved fescues belong to the taxonomically most critical groups, particularly for Southern Europe. In Italy, several authors worked on disentangling the taxonomy and systematics of fine-leaved fescues in the last decades (1). However, the *F. valesiaca* complex hasn't yet been treated. In Italy it consists of 7 naturally occurring taxa [*F. valesiaca* Schleich ex Gaudin, *F. stricta* subsp. *trachyphylla* (Hack.) Patzke ex Pils, *F. rupicola* Heuff.], 4 of which are endemic to the Alps [*F. bauzanina* (Pils) S. Arndt, *F. bauzanina* subsp. *rhaetica* S. Arndt, *F. guinochetii* (Bidault) S. Arndt, *F. ticinensis* (Markgr.-Dann.) Markgr.-Dann.]. Species of this group are charcaterized by three separate sclerenchyma strands in tiller leaf cross section (*F. valesiaca*, *F. rupicola*) which in some species can tend to conflate and form even an unevenly thickened sclerenchyma ring (*F. stricta* subsp. *trachyphylla*). They are widely distributed across the national territory and popular in restoration ecology due to their durability. However, species boundaries and identity of some populations remain unclear for Italy. The insufficient knowledge of this group in Italy and its wider distribution is evidenced by the recent discoveries of populations of taxa outside of their known range (2) and few endemic taxa for the Alps (3).
Our aim is to review the *Festuca valesiaca* complex in Italy and improve the knowledge on species identity and their distribution within the territory using an integrated taxonomic approach. After a complete literature review of nomenclature, both live and herbaria specimens will be morphometrically characterized and the obtained data will be analyzed via multivariate statistics. We will also make a survey of the ploidy levels, count chromosome numbers and perform genetic analysis (plastid and nuclear markers) for selected populations. We hope this will help define boundaries between individual taxa and facilitate their more informed use in restoration ecology. An *ex-situ* collection of seeds representing the diversity accounted for in our study will be realized and preserved in the germplasm bank of the University of Pavia. The seeds collection combined with a better understanding of the group will serve as a basis to further improve the use of the different taxa in habitat restoration activities.

1) B. Foggi, G. Parolo, P. Šmarda, A. Coppi, L. Lastrucci, D. Lakušić, G. Rossi (2012) Bot. J. Linn. Soc. 170, 618-639.
2) G. Filibeck, L. Cancellieri, F. Bartolucci, U. Becker, F. Conti, S. Maestri, M. Mürz, E. Schommer, M.G. Sperandii, T. Becker (2019) Pl. Biosystems 154(5), 593-600.
3) S. Arndt (2008) Pl. Syst. Evol. 271, 129-142.

## 2.6 = Integrative species delimitation in *Xanthium* sect. *Acanthoxanthium* (Asteraceae)

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*Xanthium* L. is a peculiar genus of the Asteraceae, characterized by spiny, wind-pollinated, female capitula (burs). The burs are also important morphological characters used to delimit taxa in the genus. Due to their extreme variability, taxonomic treatments based solely on morphology have often failed unequivocally recognising borders among species.
Two sections are recognisable in the genus: sect. *Xanthium*, including all taxa with unarmed stems; and sect. *Acantoxanthium* DC, with plants characterized by stems bearing trifurcate spines. Taxa of sect. *Acanthoxanthium* are native of South America and some of the lineages has reached nowadays a cosmopolitan distribution thanks to human mediated dispersal. *Xanthium spinosum* L. indeed was brought accidentally to Europe during the XVII century and is currently considered an important weed in large part of the Globe.
A part for the widespread *X. spinosum*, several taxa have been described in the section. Widder (1923) recognised five species in the section and some infraspecific taxa within *X. spinosum*. Some of the species were described based on single herbarium vouchers.
In the present study, we aim at delimiting species in *Xanthium* sect. *Acanthoxanthium*. For the scope, we used phylogenomics (target enrichment of a few hundreds of nuclear loci and complete plastomes) and geometric morphometrics, along with coalescent-based species delimitation approaches. We surveyed some of the most important herbaria (e.g., B, BA, WU, P) to sample a broad range of the morphological variation observed in the section, including types and original material for some of the taxa.
The analyses show that four independent evolutionary lineages are recognisable in the section. Beside the two species already recognised in (1), i.e., *X. spinosum* and *X. ambrosioides* Hook. & Arn., two additional lineages are found as independent species (i.e., *X. catharticum* Kunth and *X. argenteum* Widder. Interestingly, *X. argenteum* was described by (2) based on a single herbarium voucher collected in the historical province of "Nubes" in Chile. We were able to find another herbarium specimen ascribable to this species in Paris (P). *Xanthium catharticum* turned out to be well-separated from *X. spinosum* and represents its vicariant in the high-mountains environments of the Andes.

Fig. 1. Type specimen of *Xanthium argenteum* housed in WU.

Fig. 2. Specimen of *X. argenteum* found in P.

1) S. Tomasello (2018) Molecular Phylogenetics and Evolution, 127, 135-145.
2) F.J. Widder (1923) Repert. Spec. Nov. Regn. Veget., 20, 1-223.

## 3.1 = Carpological analysis of a Roman well at the site of *Alba Fucens* (Abruzzi, Italy)

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Abruzzo is, after Valle d'Aosta, the second least investigated Italian Region in terms of archaeobotanical analyses, with only four sites published so far (1, 2). Of these, only one is of Roman age (3).
In this perspective, the archaeological site of *Alba Fucens* (L'Aquila) represents a great addition to the state of the art. Presumably founded by the Equi, an Italic tribe, *Alba Fucens* (Fig. 1) became an important Roman colony at the end of the 4th century BC. Inscriptions testify its prosperity during the Imperial period. Its splendor ceased in 537 A.D. during the Gothic war.
The archaeological materials, deriving from years of excavation campaigns led by the "Soprintendenza per i Beni Archeologici dell'Abruzzo", are until now poorly investigated from a scientific perspective. The present study focuses on plant remains recovered from a Roman well. Located in the square of the Sanctuary of Hercules, the well was identified during the excavations of 2011. It is possible to suppose that the filling of the well is the result of an action after the destruction of the sanctuary of Hercules following an earthquake in the Late Roman period. Part of the collapsed materials was moved into the reservoir, to recover practicable space in the square of the sanctuary, located along the Via Valeria in a lowered and protected position.
The archaeobotanical analysis was performed on six soil samples (total of 12.6 kg), collected from six different Stratigraphic Units (SUs) from the filling of the well. The progressive numbering of the SUs indicates a greater depth with respect to the planking level. Separation of plant macro-remains was performed by wet sieving at the *Museo delle Paludi* in Celano (Soprintendenza Archeologia, Belle Arti e Paesaggio per le Province di Chieti e Pescara).
More than 1500 carpological remains preserved by waterlogging were identified in the studied sediment. These are represented by 70 different taxa, belonging to vascular plants and mosses. Carpological remains are mostly attributable to gathered fruit plants (e.g. *Corylus avellana* L., *Juglans regia* L. and *Sambucus nigra* L.) and spontaneous herbaceous species (e.g. *Marrubium vulgare* L. and *Polygonum rurivagum* Jord. ex Boreau).
Overall, the assemblage describes Apennine grasslands, influenced by human presence. The study also provides precious information about the diet of the Roman inhabitants of the site and the exploitation of the natural resources that the surrounding environment had to offer.

Fig. 1. The archaeological site of *Alba Fucens.*

1) M. Mariotti Lippi, A. Florenzano, R. Rinaldi et al. (2018) Flora Mediterranea, 28, 365-376.
2) A.M. Mercuri, E. Allevato, D. Arobba et al. (2015) Rev. Paleobot. Palynol., 218, 250-266.
3) C. Shelton (2009) Food, economy and identity in the Sangro River Valley, Abruzzo, Italy, 650 B.C. – A.D. 150. Boston University, PhD dissertation.

## 3.2 = GeneBank Molecular Dataset for Autochthonous Plants: Which is the Availability Rate? The case of Monterozzi SCI Site

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DNA barcoding has become the primary tool for critical plant species identification, becoming in the last decades crucial in many fields even distant from each other such as biomonitoring and food fraud. Recently this method has been applied for not-lignified root identification for the preservation of underground built heritage [1]. Vegetation plays, indeed, a dual role in archaeological sites. Plants contribute significantly to the characterization of landscapes, enhancing their naturalistic and ecological value, but vascular plants, generally trees, can seriously threaten the conservation of ancient monuments, especially hypogeal, as they can damage the structures by root expansion. Species identification is then crucial for an effective control of the vegetation in archaeological sites and to define a management plan and it should be fast and reliable even if the available specimens are immature, ruined or lack one or more fundamental characters for their morphological identification. Plant DNA barcoding represents the solution even if some difficulties arise from their three different genomes (i.e., nuclear, plastidial, and mitochondrial), and from the variable distribution of organelles by tissues and age [2]. In a previous work, focused on herbaceous roots [1], we highlighted the need of enlarging the reference dataset with attention to meso-Mediterranean herbaceous wild species. With this purpose, using the check list of the Monterozzi archaeological area and SCI/SAC (IT6010028) site [3], we performed a review of the GeneBank database to measure the data availability relative to the autochthonous flora. The search involved the 177 SCI/SAC identified species, distributed in 131 genera and 39 families. GenBank records were screened for the most used barcode markers, namely ITS, matK, rbcL, trnH-psbA, checking also the sequences length and the availability of complete plastid genome. The most represented GenBank marker is ITS covering the less represented was the intergenic spacer psbA-trnH, that present the highest incidence of successful sequencing in our last work. The preliminary results highlight that the common species, and in particular species with an applicative interest, are usually represented, but a great lack exists for the rare or endemic species. For example, the species with conservationist interest, such as *Ajuga iva* (L.) Schreb., *Centaurea nutans* L., or *Linaria purpurea* (L.) Mill. are few represented with ITS, MatK and rbcl, but the complete chloroplast genome and the psbA-trnH lack. At last, most of the sequences are not vouchered by an institution and/or by collections that guarantee their identification. This study is very important for the management and conservation of archaeological sites as much as for the conservation and management of the SCI itself. However, in broader terms it wants to be a starting point for a broader analysis at regional and national level, to be able to give a focus on the data to be integrated and to create a specific database.

1) D. Isola, F. Bartoli, S. Langone, S. Ceschin, L. Zucconi, G. Caneva (2021) Plants, 10(6), 1138.
2) K.A. Lutz, W. Wang, A. Zdepski, T.P. Michael (2011) BMC Biotechnol., 11, 54–59.
3) G. Zangari, F. Bartoli, F. Lucchese, G. Caneva (2023) Biodiversity and Conservation. (under review).

## 3.2 = Digitalization of secondary metabolites produced by the flora of an Integral forest of Po Valley: a successful strategy for the identification of novel active metabolites

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Biodiversity is one of the most underestimated priceless wealth of the Earth. The exploitation of the territory for agricultural, construction or touristic purposes and the improper waste handling represent an increasing threating for many habitats and species. To overcome this problem, many actions have been performed. Among the others, in 1964 the International Union for Conservation of Nature's Red List of Threatened Species (IUCN Red List) was established and evolved to become the most comprehensive database on risk status of animals, fungi and plants (1).
Another promising strategy to defend biodiversity is to institute integral nature reserve, where human management is completely forbidden. In this work, we want to highlight the value of these integral habitats in the scientific research and their importance in maintaining the biodiversity of a selected environment. With this aim, the subject of our work has been Bosco Siro Negri, a small strip of the Po Valley that was donated to the University of Pavia in 1967.
The area is located in northern Italy, about 15km from Pavia (Lombardia), on the right bank of Ticino river, and belongs to the larger Site of Community Importance IT 2080014 "Bosco Siro Negri e Moriano", which covers an area of 1,352 hectares (2).
To deep our knowledge about Bosco Siro Negri and to highlight its value in the medicinal chemistry field, we exploited an already existing checklist of this area and built a database of secondary metabolites produced by the species growing there. Next, we implemented this data with their classification (e.g. tannins, flavonoids, terpenoids, etc.) and associate them with their isomeric SMILES code and their IUPAC name.
Preliminary results highlighted that most of the metabolites produced by the considered species belong to flavonoid class (38%), followed by organic acids (21%) and terpenoids (12%). This data makes the Bosco Siro Negri Reserve an interesting source of biologically active molecules, being many metabolites belonging to these classes already studied for the treatment of different pathologies.
To conclude, this integral nature reserve has demonstrated to be a hotspot of pharmaceutically interesting plants and the exploitation of the DB of secondary metabolites for computational screening could represent a powerful tool to identify new active metabolites.

1) IUCN 2023. The IUCN Red List of Threatened Species. Version 2022-2. <https://www.iucnredlist.org>
2) Bosco Siro Negri. https://boscosironegri.unipv.it

### 3.2 = Lichen data in Italy: aggregation, interoperability, and integrated services

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The aggregation of biodiversity data plays a pivotal role for facing the challenges of global changes, as it allows researchers to address complex questions, gaining deeper insights on biodiversity patterns and ecological dynamics.
As far as lichens are concerned, ITALIC, the information system on Italian lichens (https://italic.units.it) aggregates information and resources about the taxa known to occur in Italy, as well as a selection of taxa which should be looked for in the country.
Since its creation, the system has evolved, becoming an aggregator of a wealth of nomenclatural, systematic, distributional, and ecological data. Furthermore, in the last 2 years, novel services were created, among which a name-matching tool to align scientific names to those accepted in the national checklist, and digital identification keys covering practically the whole lichen flora of Italy.
Recently, ITALIC also started to aggregate occurrence data from herbaria, as well as from floristic-vegetational surveys and literature, for a total of about 140,000 records. All occurrence data are available under CC-BY license, thus allowing researchers to download and reuse them by properly citing the source. For increasing interoperability, data are made available in Darwin Core standard format. This also permits their aggregation in the Global Biodiversity Information Facility (GBIF), which was already achieved for ca. 20% of records.
As a further service to the research community, most data are now accessible by means of standalone application programming interfaces (APIs), or via a package available in R and python. This allows the seamless integration of ITALIC tools and data into any research workflow.
By incorporating modular components and standardized data formats, the structure of the system has been designed to be replicable, and can be effectively adopted for aggregating biodiversity data across other taxonomic groups as well.

## 3.2 = 2013–2023: Ten years of Wikiplantbase #Toscana

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Since 2009, we have initiated a series of regional floristic contributions titled "*Contributions for a vascular flora of Tuscany*" in the journal *Atti della Società Toscana di Scienze Naturali, Memorie, serie B* (1). Towards the end of 2012, we recognized the need for a freely accessible platform to store floristic data in Tuscany. Consequently, the floristic records from the aforementioned series formed the initial core of the Wikiplantbase #Toscana project, which was launched as a collaborative online platform in June 2013 (2). Since its inception, the database has experienced continuous growth, in terms of both collaborators (currently 85) and floristic records (currently 404,960). This growth has also spurred the establishment of sister projects in Sardegna (http://bot.biologia.unipi.it/wpb/sardegna), Liguria (http://bot.biologia.unipi.it/wpb/liguria), Sicilia (http://bot.biologia.unipi.it/wpb/sicilia), Emilia-Romagna (http://bot.biologia.unipi.it/wpb/emiliaromagna), and a national project (http://bot.biologia.unipi.it/wpb/toscana/index.html) supported by the Working Group for Floristics, Systematics and Evolution of the Italian Botanical Society. Wikiplantbase #Toscana provides primary floristic data to GBIF (https://www.gbif.org/dataset/5445131a-b491-44d8-9974-3ec52bde0c47), where the dataset collected 480 citations. In October 2022, the website underwent a complete renovation (8). The interface has now been migrated to the Yii 2.0 framework (www.yiiframework.com), ensuring improved security and ease of navigation. An analysis of the density of floristic records currently stored in the database reveals several hotspots in northern Tuscany, Elba Island, Monte Amiata, and the Argentario Promontory (Fig. 1). Most records report the presence of native species, but also alien and cultivated plants are reported (Table 1). The most active contributors (each with over 10,000 records) are David Dolci, Brunello Pierini, Lorenzo Pinzani, Giulio Ferretti, Francesco Roma-Marzio, Giovanni Gestri, Lorenzo Peruzzi, Katia Francesca Caparelli, and Gianmaria Bonari. The most frequently recorded species are all trees (*Quercus cerris* L., *Castanea sativa* Mill., *Quercus pubescens* Willd. subsp. *pubescens*, *Fagus sylvatica* L. subsp. *sylvatica*, *Robinia pseudoacacia* L., *Ostrya carpinifolia* Scop., and *Fraxinus ornus* L. subsp. *ornus*), with the exception of the climber *Hedera helix* L. subsp. *helix* and the invasive herb *Arundo donax* L.

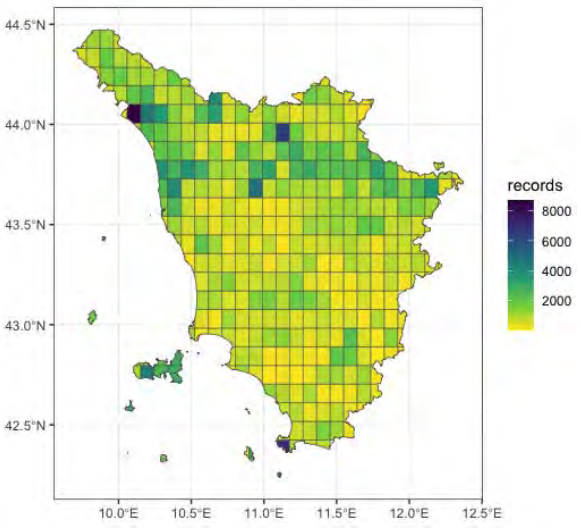


Fig. 1. Map showing the density of floristic records stored in Wikiplantbase #Toscana.

Table 1. Number of presence, doubtful, excluded records by native or alien status.

| | Presence | Doubtful | Excluded |
|---|---|---|---|
| Native | 354,446 | 1,867 | 812 |
| Invasive aliens | 9,232 | 5 | 4 |
| Naturalised aliens | 6,623 | 26 | 10 |
| Casual aliens | 1,919 | 29 | 18 |
| Cultivated plants | 29,945 | 12 | 13 |

1) L. Peruzzi, D. Viciani D., G. Bedini (eds.) (2009) Atti Soc. Tosc. Sci. Nat., Mem., ser. B, 116, 33-44.
2) G. Bedini, B. Pierini, F. Roma-Marzio, K.F. Caparelli, G. Bonari, D. Dolci, G. Gestri, M. D'Antraccoli, L. Peruzzi (2016) Plant Biosyst., 150(3), 601-610.

## 3.2 = The 'Nature Map System' project of the Calabria Region

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Calabria is one of the few Italian regions without a 'Nature Map', a tool provided for by the 'Framework Law on Protected Areas' (L. n. 394/91) to assess the state of the natural environment and to highlight natural values and territorial vulnerability profiles. To fill this gap, the regional council promoted the 'Nature Charter System' project together with the Mediterranean University of Reggio Calabria, the University of Calabria and the ISPRA, as part of the Axis 6 Action Programme - Protection and Enhancement of the Environmental and Cultural Heritage. The production of the map was realised using GIS tools starting from the 1:5,000 scale 'Map of Places' available at the Region of Calabria through photo-interpretation activities and subsequent field verifications. To classify habitats, a regional legend was developed based on the national one prepared by ISPRA (1, 2) and adapted to the specificities of the habitats occurring in the region, considering the available bibliographic vegetation data. A description, reference species, links to other classification systems (EUNIS, Habitat Directive EEC 43/92, CORINE Land Cover), reference to syntaxa, distribution in the Natura 2000 Network, and bibliographic references were provided for each habitat. The legend includes 120 habitats in 7 groups: 1. Coastal and halophytic communities (14 habitats); 2. Non-marine waters (7); 3. Scrub and grassland (36); 4. Forests (30); 5. Bogs and marshes (4); 6 Inlands rocks, screes and sands (5); 8. Agricultural land and artificial landscapes (23). Field surveys were carried out using two methods: expeditious surveys using georeferenced photos and recognition of typical habitat species, and surveys of the habitat's vegetation by a field sheet form that collects: geographic and topographic data, community structure, phytosociological relevés, pressures and threats. In total, more than 1,500 control points were carried out. The project is currently in the final verification phase of the typologies and updating of the geometries. The Nature Map of the Region of Calabria is an important territorial information system for the knowledge and mapping of biodiversity that, in addition to assessing the conservation status of ecosystems, estimating the ecological value of environmental units, highlighting critical issues at the regional scale, allows the identification of ecological connectivity areas for the effective planning of the Regional Ecological Network.

1) ISPRA (2009) Gli habitat in Carta della Natura - Schede descrittive degli habitat per la cartografia alla scala 1:50.000. ISPRA, serie MLG 49/2009 ISBN 978-88-448-0382-7.
2) ISPRA (2022) https://www.isprambiente.gov.it/it/servizi/sistema-carta-della-natura/nuova-legenda-nazio-nale-per-la-cartografia-degli-habitat [Accessed: 9/11/2022].

### 3.3 = Unraveling climate risks for plant regeneration by seeds in the Mediterranean Basin

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Seed germination studies are crucial to understanding early life cycle plant responses under current and future environmental conditions. This is fundamental to test evolutionary, ecological, and biogeographical hypotheses in plant science. Particularly, measuring seed thermal responses is crucial to identify climate risks for regeneration and can inform the implementation of seed-based solutions to environmental and societal challenges. Unfortunately, seed germination data are still scarce or disaggregated for many species and regions. This is particularly unfortunate for one main global hotspot of plant diversity such as the Mediterranean Basin, where historical and recent changes in temperature, land uses, and fire regimens make it a very challenging biogeographical region to maintain plant diversity.
Our goal is to identify native flowering plants from the Mediterranean basin that exhibit a germination niche compatible with current and future warming scenarios. To this end, we created MedGermDB, the first germination database of the Mediterranean Basin.
MedGermDB is the first effort to integrate the available germination information of the Mediterranean Basin using a new systematic approach that allows to automatically identify the literature sources with relevant information, and it uses semi-automatic tools to extract available data and quickly compile curated data sets. In total, 2837 references were screened, finding 134 articles from which data were extracted referring to key species from Mediterranean habitats as provided by EUNIS-Esy expert system, which contains definitions of individual habitats based on their species composition and geographic locations, allowing for maximising species diversity for future ecological and evolutionary plant studies. The database includes 4967 germination tests under controlled laboratory conditions varying in incubation temperature, dormancy-breaking treatments, photoperiod, and storage for 249 species, and 43 angiosperms families collected in 23 countries.
Our database comprises key functional species representative of the whole Mediterranean region, and it will be the fundamental setting to apply multilevel germination modelling accounting for species identity, phylogenetic relatedness, habitat variation, and species' uses for human well-being and conservation actions. This approach will allow to identify germination syndromes at species and above-species levels compatible with warming scenarios. MedGermDB and the associated methodology bring crucial applicabilities: (1) quick expansion and transferability to other biomes and plant lineages; (2) contributing to global efforts aggregating germination data; (3) serving as a backbone for effective seed-based conservation and climate-smart ecological restoration actions.

## 3.3 = Seed priming in *Salvia ceratophylloides*: plant development and ecophysiology and responses to combined abiotic stress

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*Salvia ceratophylloides* Ard. is an endemic and rare species, localized in the sandy hills of Reggio Calabria suburbs (southern Italy). Declared "extinct in the wild" (EW), this plant species was rediscovered during field surveys conducted in the last decade (1), and is currently fragmented into 17 micropulations (2). Several studies have been conducted to understand the biology, ecophysiology, population structure and dynamic of *S. ceratophylloides* (2). Among the most interesting bio-technologies, seed priming received more attention and interest from researchers for different reasons. It is eco-friendly and economical presowing technique that speed and allow uniform seed germination, increases the seed vigor and improves the seedling quality for the transplant operation (3). Technically, it is a pre-sowing seed treatment that allows controlled hydration of seeds that start the germination process but are stopped by re-hydration before the radicle/epicotyl emission (3). The controlled hydration of the seeds can occur in water (hydropriming) or solution of salts, PEG or mannitol (osmopriming), germination promoters (hormopriming) or living microbial cells (biopriming). This study, which focused on the phenological and ecophysiological responses of *S. ceratophylloides* to single and combined stresses showed that individuals whose seeds were exposed to priming treatments exhibited well-functioning, healthy plants that responded early and actively to simulated stresses of future climate change. The treatment positively influenced seedling emergence with a statistically significant increase [123% in the Osmopriming (OP) treatment and 108% in the Hydropriming (HP) treatment] compared to the non-primed seed (control, CP). *S. ceratophylloides* plants treated with OP and HP showed a higher net photosynthetic rate [4.16 and 3.91 μmol ($CO_2$) $m^{-2}$ $s^{-1}$, compared to 2.95 μmol ($CO_2$) $m^{-2}$ $s^{-1}$ for CP. Heat (H) and combined stress (drought + DH heat) caused a significant reduction in PN by more than 50% compared to the control. Stomatal conductance measured at all three irradiations and in the presence of heat and combined stress was statistically reduced by 84% compared to the control (C), suggesting a stomatal-dependent reduction in photosynthesis. Overall, all the ecophysiological results pointed out a synergistic rather than additive effect of the two stresses. The seed priming is very interesting for the biology of conservation by native plant species, notably characterized by low, recalcitrant and non-uniform germination process. But above all, it is fundamental for the restoration ecology of fragile ecosystems such as those predominate by no-optimal environmental conditions with raising of drought, heat, nutritional, flooding, and salt stress (3).

1) G. Spampinato, V.L.A. Laface, A. Cano Ortiz, R.Q. Canas, C.M. Musarella (2019) http://dx.doi.org/10.5772/intechopen.84905.
2) V.L.A. Laface, C.M. Musarella, A. Sorgonà, G. Spampinato (2022) Sustainability, 14, 10295: https://doi.org/10.3390/su141610295.
3) S. Paparella, S.S. Araújo, G. Rossi, M. Wijayasinghe, D. Carbonera, A. Balestrazzi (2015) Plant Cell Rep., 34, 1281–1293. https://doi.org/10.1007/s00299-015-1784-y.

## 3.3 = Propagation techniques of *Abies nebrodensis* by the side-veneer graft

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*Abies nebrodensis* (Lojac.) Mattei is the most expressive element of the Sicilian Forest flora and the Madonie Natural Park, representing one of the most considerable species to be protected due to his endemic character, relict significance and serious risk of extinction. This species, included in Annexes II and IV of the Habitats Directive (92/43/EEC), is critically endangered according to the I.U.C.N. classification. For this reason, over the last 20 years, two LIFE Nature projects have been implemented (n° LIFE2000NAT/IT/7228 and LIFE18 NAT/IT/000164), with the aim of removing or limiting the endangered conditions of the species by implementing both *in situ* and *ex situ* conservation actions.

For the *ex-situ* conservation and to preserve the genetic heritage of the 30 mother trees, a clonal orchard was created within the Piano Noce Regional Forest Nursery in the municipality of Polizzi Generosa using the grafting propagation. Scions were taken from the mother plants of *A. nebrodensis,* growing in the native range at the end of winter, soon before the beginning of the growth cycle during the vegetative rest period. The field activity was carried out on three consecutive days (29, 30 and 31 March 2022), taking scions from plants no. 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22, 26, 27, 28, 29, 30, 32. Due to adverse environmental conditions and/or the absence of suitable plant material, plants no. 2, 15, 21, 23, 24, 25, 31 were grafted in April 2023.

The entire process was carried out following a specific protocol. Scions were harvested by selecting the most vigorous branches of the last growing season, free of floriferous buds, with a cross-section of no less than 5 mm. Harvesting was carried out on primary branches at a height of approximately 4 m, topping the primary branch for a maximum of 10-20 cm and releasing part of the branch. This technique will allow the underlying bud to resprout and the cut branch to continue its growth. The material collected was labelled with the initials of the mother tree of origin, placed inside plastic bags and stored at +4° C at the Piano Noce RFN. The number of scions harvested from each plant was different in relation to the different ecophysiological and structural conditions of the individuals.

Grafting was carried out the day after harvesting.

The rootstocks used were between 10 and 15 years old and at least 5 mm in diameter. They came from *A. nebrodensis* open pollinated seedlings. The side-veneer grafting procedure was mainly used and only in few cases double side-veneer grafting was followed.

The side-veneer graft consists of joining the scion to the rootstock without cutting it. Each scion was prepared according to the typical guidelines of side-veneer grafting, while a longitudinal shaping of the same width as that made on the scion was carried out on the rootstock, after removing the needles on a portion of the stem at least 5 cm long, also making a small wedge at the base of the cut to favour the adhesion of the two bionts.

The scion, after being inserted inside the wedge by matching the vascular cambium of the two bionts, was tied with elastic grafting bands and covered with healing mastic. The graft was then wrapped with aluminium paper and covered with a PE bag. For the year 2022, the total number of grafts performed was 454, and the average rate of living grafts was around 33% after one year. Such a high success rate has never been achieved in the grafting of *A. nebrodensis*.

### 3.4 = Relationship between beach litter amount and distribution and psammophilous habitats in a Mediterranean coastal dune system

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One of the most common pollution issues affecting coastal areas worldwide is the presence of beach litter. This anthropic component is defined as a portion of marine litter, meaning "any persistent, manufactured, or processed solid material discarded, disposed of, or abandoned in the marine and coastal environment"; this is a complex issue that has global implications for human activities in addition to marine and coastal ecosystems (1). The main objective of this study was to evaluate the relationships between the abundance and distribution of beach litter and the different psammophilous habitats on a Mediterranean sandy beach (Porto Paglia, Sardinia, West Mediterranean Basin). To this end, two seasonal sampling surveys were conducted during the period spring–autumn 2022. A total of 39 plots were randomly placed and surveyed across the entire psammophilous vegetation zonation, from the unvegetated area to the backdune. For each plot, the overall plant coverage and the relative coverage of each vascular plant have been visually evaluated; subsequently, each plot was assigned to a specific habitat of EU interest. In addition, in each plot the superficial beach litter was counted by viewing only the macrolitter (objects > 25 mm in the longest dimension) and the mesolitter (objects between 5 mm and 25 mm in the longest dimension) (2) and categorised according to the most recent European manual (2). Additionally, each plot was photographed to provide orthogonal digital images that were subsequently used in laboratory to determine each object's size. In the study area four different habitats were identified: annual vegetation of drift lines (Habitat 1210), embryonic shifting dunes (Habitat 2110), shifting dunes along the shoreline with *Ammophila arenaria* (white dunes, Habitat 2120), and *Crucianellion maritimae* fixed beach dunes (Habitat 2210). During the two samplings, a total of 614 objects were collected, 324 of them in autumn and the rest in spring. As previously demonstrated, our results confirm that the main beach litter category is plastic, with 192 objects in spring and 225 in autumn; in particular, the most numerous items were plastic items and fragments, foamed polystyrene fragments, foam rubber fragments, and plastic caps (3). Our results show that beach litter distribution varies across habitats; in particular, we found that Habitat 2120 seems to play a greater role in trapping and filtering beach litter, reducing its amount in the backdune (3). This finding is supported by the fact that in Sardinia, more than elsewhere, this habitat is still well conserved. However, a seasonal trend in the amount and distribution of beach litter in relation to psammophilous habitats was observed: in spring, Habitat 2120 was the richest in terms of number of objects, and Habitat 2210 was the poorest, while in autumn, Habitat 1210 was the richest in terms of number of objects, and Habitat 2210 was the poorest. This pattern could be influenced by summer tourism as well as winter storms. However, our results are preliminary, and the same analysis will be repeated in other Sardinian dune systems and at different periods to investigate how beach litter varies across habitats and the role of touristic exploitation. The widespread presence of beach litter is one of the most important issues affecting Mediterranean coastal areas; consequently, the presence of this ecological component should be considered in the conservation status assessment of a coastal dune systems. Hence, the analysis of this ecological component could be useful to identify sustainable management measures for this fragile ecosystem.

1) UNEP (2009) Marine Litter: a Global Challenge. UNEP, Nairobi (232 p.).
2) D. Fleet, T. Vlachogianni, G. Hanke (2021) A Joint List of Litter Categories for Marine Macrolitter Monitoring. EUR 30348 EN, Publications Office of the European Union, Luxembourg.
3) G. Calderisi, D. Cogoni, A. Loni, G. Fenu (2023) Marine Pollution Bulletin 192, 115065. https://doi.org/10.1016/j.marpolbul.2023.115065

## 3.4 = Biodiversity conservation through robotic field monitoring

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One of the most important tools for biodiversity conservation in Europe is the Natura 2000 network, as defined by the European Council Directive 92/43/EEC. Each EU member state is in charge of the network's periodic habitat monitoring, which typically comprises the floristic and structural characterization of vegetation to evaluate its functionality. At present, only highly qualified and skilled human operators are capable of performing this work.

The H2020 Project "Natural Intelligence for Robotic Monitoring of Habitats - NI " ("Research and Innovation boosting promising robotics applications") aims to assist humans in the most repetitive and time-consuming monitoring tasks. In particular, the main project objective is to develop robots able to autonomously navigate in unstructured outdoor environments to assess the conservation status of habitats. In this abstract, we present the potential benefits of using this technology in habitat monitoring, and in particular a specifically developed quadruped. The reason behind the choice of ground robots rather than flying drones is that they can carry batteries with more capacity, resulting in a longer operation time, and they can better withstand unfavorable weather conditions such as wind or precipitation while being able to move within woods and not just above the canopy. Among the ground robots, legged systems present higher mobility and traversability capabilities w.r.t. wheeled systems.

Given these premises, we chose ANYmal C (Fig. 1), which is equipped with a LiDAR sensor and full-HD RGB-D cameras. These sensors are used to gather information on the environment, which is then passed to specifically designed classification algorithms to evaluate structural (i.e., the breast-height diameter of the trees, the vegetation cover, etc.) or floristic (i.e., the presence/absence of typical or early warning species) parameters useful to assess the conservation status of the specific habitat under analysis. The results of this procedure are promising, and we believe this solution could facilitate and boost up human operations during habitat monitoring. The data so far acquired by the robot are already publicly shared on the Zenodo platform for everyone to check and use. With the experience gained in the project, the national guidelines for habitat monitoring will be updated by integrating these new technologies, offering the international scientific and institutional communities a reference of innovation on habitat monitoring.

Fig. 1 ANYmal C robot in a grassland habitat.

### 3.4 = Environmental DNA to test the functionality of hedgerows in a vineyard system

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This study aims to assess whether landscape complexity positively influences the natural control of pathogenic arthropods in the vineyard matrix using Environmental DNA (e-DNA) tools.
The study focuses on vineyard landscape of the Telesina Valley (Southern Italy). The area is of extreme interest for agriculture economy, with over 30% of the surface area cultivated with grapevines and very low industrial land cover.
The investigated area was delimited by a 10 $km^2$ grid with a 500 m pitch, located between two Regional parks (the Taburno Camposauro Park and the Matese Park). With an initial random selection, the vineyards were classified into 4 groups according to a level of naturalness, which correspond to the percentage of the perimeter covered by stable and persistent vegetation (hedgerows). Tree and/or shrub vegetation with a thickness of at least 2 m and continuously surrounding the edge of the selected vineyard were considered. We hypothesised that the more composite the hedge, the greater the biological reservoir and the possibility of attracting different species. This biodiversity stabilises the ecosystem and crop auxiliary plants can become allies against pests.
We applied DNA metabarcoding with the aim of exploring how the diversity of arthropods can be affected by different degrees of naturalness of the vineyard system. Leaves of Vitis vinifera and leaves and/or floral elements of spontaneous vegetation surrounding the vineyard were taken from 5 vineyards of the three groups. To detect all species in a sample this untargeted approach is used, called 'metabarcoding'. E-DNA is analysed through Next generation sequencing (NGS).
The results of the analysis indicate the presence or absence of DNA of species or particular groups of species (in this case arthropods). DNA can also be quantified and linked to species abundance.
These results will be correlated with those from two forest areas of high ecological value (Taburno Camposauro Park and the Matese Regional Park) that are continuously connected to our wine-growing agroecosystem.
We expect biodiversity to vary in relation to the ecological network and thus to be higher in the core areas and patches of high ecological value, and to decrease in buffer strips and primary connection areas (agrarian landscape). Furthermore, we expect the increasing degree of naturalness in the primary connection areas (vineyard area) to reflect the trend in alpha diversity of the entire ecological network.
Overall, our study (i) reiterates the potential of eDNA techniques as a tool for assessing how biodiversity is affected by agricultural management which strongly influence ecological network, and (ii) can provide new knowledge aimed at guiding sustainable land use management decisions.

### 3.4 = Using remote sensing to map habitat mosaics: an integrated approach applied to the classical Karst

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Remote sensing is a well-established tool for habitat mapping, but its use is still challenging in heterogeneous landscape mosaics. Novel approaches to improve classification performance include multi-temporal data and multiple remotely sensed variables, but have rarely included spectral heterogeneity (SH) measures. The aim of this study was to develop an integrated approach to map the natural habitats in Classical Karst (NE Italy), by quantifying the importance of SH measures and providing a robust framework to include multi-temporal remotely sensed data.

First, a habitat map was produced from field surveys. Then, a collection of 12 monthly Sentinel-2 images was retrieved using the Google Earth Engine platform. Vegetation and SH indices were computed and aggregated in four temporal configurations: (1) monthly layers of vegetation and SH indices; (2) seasonal layers of vegetation and SH indices; yearly layers of multi-temporal SH indices computed (3) across the months, and (4) across the seasons. For each temporal configuration, a Random Forest classification was performed, first with the complete set of input layers and then with a subset obtained by Recursive Feature Elimination. Training and validation points were independently extracted from field data.

The maximum overall accuracy (OA = 0.72) was achieved with the seasonal temporal configuration, after the number of habitat classes was reduced by aggregation from 26 to 11. SH measures allowed to improve the accuracy of the classification and the spectral $\beta$-diversity was the most important variable in most cases. Spectral $\alpha$-diversity and Rao's Q, on the other side, had a low relative importance, possibly due to the small spatial extent of the habitats. Regarding the inclusion of multi-temporal data, the aggregation of monthly data in seasonal median composites proved to be the best approach, since it allowed to reduce the number of input layers without losing accuracy.

The approach developed in this study allows to improve habitat mapping in complex landscapes in a cost- and time-effective way, suitable for monitoring applications. Moreover, the results suggest that image classification frameworks could benefit from the inclusion of SH measures, that have rarely been included before.

## 3.5 = The new *Herbarium Regium Modoetiense* of the Royal Villa of Monza

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Luca Ghini (1500-1566) was one of the first botanists who produced herbarium samples as a method for plant conservation and cataloging (1). Monza Park and the Royal Gardens constitute a wide area rich in plant biodiversity of which it is essential to keep track. At present, the Royal Villa, as the lead institution, together with its partners *Rete degli Orti Botanici della Lombardia* and *Rete dei Giardini Storici* (ReGiS), obtained in 2021 the funding of the Scienza Viva project - "*Scienza Viva. Orti botanici e giardini di delizia. Antichi saperi e nuove pratiche per la diffusione della cultura scientifica*" (Botanical Gardens and Gardens of Delight. Antique knowledge and good practices for the diffusion of scientific culture) - from the Ministry of University and Research (MUR), as part of the initiatives for the dissemination of scientific culture.
Currently, the only documents treating plant diversity are the three *Catalogus Plantarum* drawn up by Luigi Villoresi (1813), Giovanbattista Rossi (1826) and Giuseppe Manetti (1846), respectively (2). In this framework, the aim of this project consists in the realization of the first *Herbarium Regium Modoetiense* of the Royal Gardens and Monza Park, including the Royal Villa's ancient *Citrus* and *Rosa* cultivars from the Royal Villa gardens. The Natural History Museum of Milan and the University of Milano-Bicocca (Department of Environmental and Earth Sciences) are cooperating to produce the new exsiccata collections. Hundreds of samples are being collected during a 12 months period in double-copy of which half will be conserved at the Royal Villa and the other at the Museum. A digitized copy and some specimens will be retained at the Milano-Bicocca University. Until now 560 specimens afferent to 280 taxa have been collected.
This work will document the plant biodiversity in Monza's territory and it will be useful to evaluate its natural heritage. Also, if compared to past botanical collections, it can give important information about global climate change and the long-term effects on plant communities considering four crucial factors: pollution, habitat, climate and invasive species. Furthermore, the herbarium will become part of the new botanical section of the Royal Villa that the project aims to create. It will be accessible to the public to fulfill the project's goal of bringing citizens closer to science and to the benefits that biodiversity of urban green areas provides.

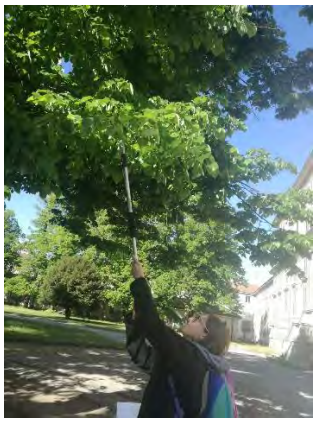
Fig. 1. Tree species sampling


Fig. 2. Herbarium specimen

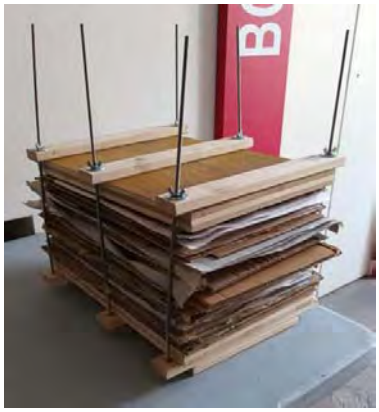
Fig. 3. The press

1) F. Taffetani (2012) Herbaria il grande libro degli erbari italiani, Nardini Editore, 814.
2) F. Pizzoni (2014) Il Parco, la Villa, Quaderno 7, 58-66.

## 3.5 = Biagio Bartalini's herbarium: a floristic archive of Siena biodiversity in the 18th century

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Italian physician and botanist Biagio Bartalini (1750-1822) was a lecturer at the University of Siena, director of the University botanic garden and a leading member of Academy of Fisiocritici. He was the first to catalogue the vascular flora of the city of Siena. He collected vascular plants, bryophytes, lichens, fungi and algae around the town and created an herbarium, which is currently kept at the Academy of Fisiocritici. In 1776 he published a catalogue "*Catalogo delle piante che nascono spontaneamente intorno alla città di Siena"* ("Catalogue of spontaneous plants around the town of Siena") of all the species he had collected. Over the years, many authors have studied this herbarium, one of the oldest in the world, but the entire collection has never been revised.
In this study we revised the sample identifications and created a database and digital archive of all the specimens. The database contains the following information on each sample: Bartalini's identification, other authors' revisions, current species name and conservation status, locality, habitat and a high-resolution photograph. The herbarium contains 568 specimens of vascular plants, 76 bryophytes, 29 lichens, one fungus and one alga. The revised specimens belong to 86 families, 325 genera, and 524 species. The collection includes 23 taxa alien to Italy, mostly crop and ornamental archaeophytes (12 species). There are also some rare species recorded before 1950 or never reported from the province of Siena according to Wikiplantbase Toscana (1). Among the herbarium samples, 25 species are included in European, Italian and Tuscan Red Lists, while 13 species are in the European list of threatened arable plants. Bryophytes include 12 liverwort samples (both thalloid and foliose) and 57 moss samples; most are species common to rocky habitats, forest floors and open mineral soils. There are also species typical of mountain areas, rivers and calcareous springs. The 29 samples of lichens include 8 species that are currently considered rather rare (2 species), rare (3 species) and extremely rare (3 species) in Tuscany; most have fruticose or foliose habitus, and many are now classified as species of mountain habitats.
Study of the herbarium revealed important information about the flora of the past, including species of conservation concern such as endemic, wetland and segetal taxa that are no longer found due to urban expansion. The results provide a basis for future resampling and the opportunity to discover how the biodiversity of the study area has changed in the last 200 years under climate change and variations in land use.

1) Portal to the Flora of Italy (2020 onwards) Portale della Flora d'Italia/Portal to the Flora of Italy. 2020.1. https://dryades.units.it/floritaly/.

## 3.5 = Over a century of floristic studies in the Santa Gilla Lagoon: updates, trends and conservation implications

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The Santa Gilla Lagoon (southern Sardinia, Italy) is a complex wetland system covering an area of about 1300 ha, shaped by several wet environments of different sizes, from purely freshwater to hyperaline. To the south, the lagoon opens onto the sea through a channel, while it receives freshwater inflows from three main rivers, Riu Mannu, Cixerri and Santa Lucia. Associated with the large lagoon basin are the pond of Capoterra and one of the most important salt pans still active in Italy (Saline Conti Vecchi), while along the margins are present innumerable minor marshy areas. For its high biodiversity, it has been recognised as a Special Protection Area and Special Area of Conservation under the Council Directives 79/409 CEE and 92/43 CEE, and as a wetland site of international importance under the Ramsar Convention since 1976. The first floristic list for the area is to be attributed to Casu (1), who reports 539 vascular plant taxa for the site. De Martis et al. (2) define a list of 466 taxa, noting the failure to find many rare and/or endangered taxa, but highlighting the increase in the non-native component. Here we present first results of intensive field investigations from 2008 to present that have been carried out to review the floristic knowledge of Santa Gilla, confirming the presence of over 800 taxa, many of which are endemics [e.g. *Delphinium longipes* Moris, *Limonium avei* (De Not.) Brullo & Erben, *Plagius flosculosus* (L.) Alavi & Heywood, *Scorzoneroides muelleri* (Sch.Bip.) Greuter & Talavera] and/or of conservation [e.g. *Butomus umbellatus* L., *Halocnemum cruciatum* (Forssk.) Tod., *Malva lusitanica* (L.) Valdés subsp. *lusitanica, Nymphaea alba* L.], biogeographical [*Erodium salzmannii* Delile, *Mandragora autumnalis* Bertol., *Marrubium alysson* L., *Spirobassia hirsuta* (L.) Freitag & G.Kadereit, *Suaeda splendens* (Pourr.) Gren. & Godr.] interest. On the other hand, a significant increase in the exotic component emerged, with 47 non-native neophyte taxa counted, some of which [*Acacia saligna* (Labill.) H.L.Wendl., *Agave americana* L., *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn., *Cotula coronopifolia* L., *Hydrocotyle ranunculoides* L.f., *Lycium ferocissimum* Miers, *Parkinsonia aculeata* L., *Pontederia crassipes* Mart., *Solanum eleagnifolium* Cav., *Vachellia karroo* (Hayne) Banfi & Galasso] are invasive in most of the areas. The widespread of non-native species is produced by anthropic pressures which has characterised the history of the site since the second half of the 1900s (industries, port and airport settlements, pollution, and reclamation for agricultural and urbanization purposes). In 2023, eradication actions and consequent renaturation of habitats were implemented through the translocation of native taxa (Project financed by Metropolitan City of Cagliari through European funds within the Prioritized Action Frameworks (PAFs) to implement the EU-wide Natura 2000 network (action 6.5.1 POR FESR 2014-2020). These results are of crucial importance for updating knowledge and provide the basis for the development of necessary further studies and conservation actions.

1) A. Casu (1911) Nuovo Giorn. Bot. Ital., 3:363-415.
2) B. De Martis, A. Marchioni, E. Bocchieri, A. Onnis (1983) Atti Soc. Tosc. Sci. Nat., Mem., Serie B, 90:149-255.

## 3.5 = An update on the flora of the Lagoon of Capo Peloro (north-eastern Sicily, Italy)

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The natural reserve of the Lagoon of Capo Peloro is located in north-eastern Sicily (Italy) and it consists of two euryhaline ponds, Lake Faro and Lake Ganzirri. Communication with the sea takes place through natural and artificial channels; the latter frequently tend to get covered up by sand (1, 2). Both lakes are connected by a canal (Margi), which was once a shallow marsh, reclaimed by human action in historical times. Despite the very strong urbanization that surrounds the lakes has made part of their shores totally cemented, nevertheless both lakes are characterized by a rich vegetation: there are spontaneous halophytic, helophytic, riparian, ruderal and exotic species, mostly introduced by man, which are well adapted (3). Among riparian species, the family *Poaceae* dominates essentially with three species, namely *Phragmites australis* (Cav.) Trin. ex Steud., *Arundo donax* L. and *Saccharum biflorum* Forssk., which give rise to compact and monospecific formations in various associations (*e.g.*, *Phragmition communis* Kock and *Convolvulion sepium* Tüxen). Peculiar plant communities in Lake Ganzirri are one dominated *Cyperus alternifolius* L., and one characterized by *Washingtonia filifera* (Gloner ex Kerch., Burv., Pynaert, Rodigas & Hull) de Bary, exotic plants that have been naturalized since long time along the northern shore. Noteworthy, *Cynanchum acutum* L., a rare paleosubtropical species of euryhaline lake environments, is only present in Lake Faro. Among aquatic species, it should be noted the presence of allochthonous macroalgal species introduced by the importation of molluscs, some of which with invasive behavior (for example, *Ulva* spp. and *Hypnea* sp.) especially in Lake Ganzirri, but also of native species with ecological significance for the associated biological communities, such as the angiosperm *Cymodocea nodosa* (Ucria) Asch. and the calcareous algae of the family *Corallinaceae*.

1) T.L. Maugeri, C. Gugliandolo, D. Caccamo, T. La Rosa (1999) IX Congresso Nazionale Società Italiana di Ecologia, Lecce.
2) F. Lo Coco, F. Lanuzza, G. Adami, G. Cappellano, F. Cozzi, F. Mondello (2006) Atti del "XXII Congresso Nazionale delle Scienze Merceologiche", Roma, Edizioni KAPPA, ISBN 88-7890-747-2.
3) F. Mondello, V. Pinizzotto, L. Cammarata (2000) 95° Congresso della Società Botanica Italiana, Messina.

### 3.5 = DataLake: contribution to the floristic knowledge of central-southern Italian Lakes

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Lakes cover only a small area worldwide but harbor high levels of biodiversity and provide essential and valuable ecosystem services to human existence and economy (1). Nevertheless, lakes are severely threatened by numerous phenomena, such as climate change, eutrophication, water abstraction, water-level regulation, hydro-morphological alteration, resource exploitation, and biological invasions (2). In such a dynamic and vulnerable environment, it is crucial to adequately characterize the biodiversity to understand the ongoing processes and enable timely management interventions. To this aim, a floristic database was developed to collect and catalog plant data available in literature to define and update the floristic knowledge of natural lakes in Italy (DataLake). In particular, lakes of central-southern Italy of volcanic origin, well represented here, and lakes of glacial origin, often overlooked by the floristic investigations, are prioritized in this study. To maximize the usability and functionality of the database, it was standardized according to the structure proposed by Global Biodiversity Information Facility (GBIF) (3) based on 'Occurrence Dataset' type of biobank. Specifically, the database provides a set of data in which for each floristic record is associated a series of descriptive fields for adequate temporal, spatial and ecological contextualization of the record (i.e., taxon, date, geographical coordinates, locality, elevation, phenological phase, Corine Land Cover, conservation status of the site, pressure and threats, bank grain size). At present, the bibliographic research is completed and the transfer of literature data into this database is in progress.
Based on previous bibliographic research this database will be implemented by original floristic data that will be collected through sampling campaigns starting with less investigated lakes. The floristic sampling will focus on the aquatic and bank environment according to an extensive approach for smaller lakes (e.g., Lake Monterosi, Lake Mezzano, Lake Duchessa), whereas for the larger ones (Lake Bracciano, Lake Bolsena) the sectors to be surveyed will be selected based on the prior knowledge, ecological representativeness and accessibility.
Given the dynamic nature of the database, future integration of floristic data related to other Italian lakes of different origins could be a subsequent desirable step toward a more comprehensive view of plant diversity in Italian lakes.
This project on lake floristic diversity knowledge in Italy is supported by National Biodiversity Future Center (NBFC) to Department of Science-University of Roma Tre, funded by the Italian Ministry of University and Research, PNRR, Missione 4 Componente 2, "Dalla Ricerca all'Impresa", Investimento 1.4, Project CN00000033.

1) R.W. Sterner, B. Keeler, S. Polasky, R. Poudel, K. Rhude, M. Rogers (2020) Ecosystem Services, 41, 101046.
2) J.P. Smol (2019). Proceedings of the Royal Society B, 286(1906), 20190834.
3) GBIF.org (2023) GBIF Home Page. Available from: https://www.gbif.org [15 May 2023]

## 3.6 = From microscopic biodiversity to flora and vegetation dynamics: Palynology for monitoring, conservation, and enhancement of Italian and Mediterranean ecosystems

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The Laboratory of Palynology and Palaeobotany (LPP) of Modena is involved within Spoke 3 (*Assessing and monitoring terrestrial and freshwater biodiversity and its evolution: from taxonomy to genomics and citizen science*) of the National Biodiversity Future Center (NBFC) under the National Recovery and Resilience Plan (NRRP). The Vision of NBFC is to monitor, preserve and restore biodiversity and make it a central element on which to base future sustainable development.
Palynology and archaeobotany can support biodiversity awareness by providing useful information on the vocation and environmental sustainability of a territory at millennial scale (e.g., 1,2).
The LPP carries out two Specific Research-lines: i) Plant biodiversity from microscopic to macroscopic level: assessment of local flora in a diachronic perspective, and ii) Archaeobotany plant collections and database implementation. The LPP team focuses on the study of plant diversity in different Italian regions with the analysis of pollen, seeds, and fruits in a long-term perspective. The contexts of interest are mainly natural (e.g., Pollino National Park; Maremma Regional Park) and archaeological parks (e.g., Vulci Naturalistic and Archaeological Park). Areas of interest are also high altitude and marshy freshwater bodies with high naturalistic value. Research focus on the main changes in flora composition related to past climate events and anthropic perturbations recorded in biostratigraphic records (palynological evidence from peat bogs and lake basins). A focus on rare species (basic information on their first appearance, distribution trend over time, and current occurrence) is also carried out with image data analysis of pollen morphology. All data will implement the BRAIN-Botanical Records of Archaeobotany Italian Network database (3), with geographical and chronological/cultural breakdown for the Holocene (last 11,700 years BP).
Selected case studies of the LPP palynological research within the NBFC will be presented.

1) A. Florenzano (2019) Sustainability, 11, 404. https://doi.org/10.3390/su11020404
2) A.M. Mercuri, V. Cannavò, E. Clò, A. Di Renzoni, A. Florenzano, E. Rattighieri, D. Yoon, S.T. Levi (2020) Journal of Archaeological Science: Reports, 30, 102235. https://doi.org/10.1016/j.jasrep.2020.102235
3) M. Mariotti Lippi, A. Florenzano, R. Rinaldi et al. (2018) Flora Mediterranea 28, 365–376. https://doi.org/10.7320/FlMedit28.365

## 3.6 = Urban green development through a palynological approach

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Allergies, including those caused by pollen grains, are on the rise due to a combination of factors, including climate change, air pollution, and new pollen allergies linked to alien plants (1). Plants growing in urban green areas are at the same time sensible to climatic changes, in highly populated and possibly polluted areas. Studying the link between urban green, pollen and other allergens is very important to understand how plants can affect the health of people living in towns and near green areas.
We aim to reconstruct the changes in urban plants in the last millennia using a palynological perspective in the study-area of Reggio Emilia to learn about the pollen rain in this urban environment since the foundation of the town.
For this reason, we collected both archaeological and aeropalynological (moss) samples in the city of Reggio Emilia (Emilia Romagna, Italy). During archaeological excavation of piazza San Prospero and Vittoria Park (Reggio Emilia), 64 archaeological samples were taken, under the supervision of prof. Cremaschi and dott. Capurso as the responsible for the Soprintendenza Archeologia, Belle Arti e Paesaggio Sabap-BO. Moss sampling in the town was based on a grid with meshes of 1 km square each. Inside each square of the grid, we collected moss samples from parks and other green public areas totalling 50 samples.
Pollen extraction includes sieving and floating steps according to the method in use in the Laboratory of Palynology and Paleobotany of Modena (2). Pollen counts were at least 300 pollen from the archaeological samples and 500 from the moss samples.
The archaeological samples cover the period from around the Roman founding of the city of Reggio Emilia to the present days. Pollen data describe a very open area (with less than 20% arboreal pollen on average) with evidence of strong human presence (22% mean API pollen, (3)) and cereal pollen possibly due to its transport and selling inside the city.
Data obtained from moss samples describe a different environment. Arboreal pollen is abundant (around 70% on average) and anthropogenic pollen are low. This suggests a relatively low presence of herb plants, possibly due to regular mowing of weeds in the parks, and a high presence of trees like ornamentals in green areas and parks of Reggio Emilia.
Allergenic pollen is present in both archaeological and moss samples. Examples of plants well represented in past and present contexts are: *Alnus*, *Betula*, *Corylus*, *Pinus, Plantago*, and *Urtica*. *Juniperus* and *Platanus* are present only in the moss samples and pollen like *Ambrosia* was mostly found in the archaeological samples.
The ongoing reconstruction of the taxa present in the town of Reggio Emilia has so far allowed for the start of the reconstruction of the history of plants in the urban green. Analysis of the moss samples supports the flora observed in the parks of the city. First results are promising to reconstruct the transformations of urban green areas and gain more information on the presence of plants with known allergenic effects.

1) A. Damialis, C. Traidl-Hoffmann, R. Treudler, Climate change and pollen allergies. In: Marselle M, Stadler J, Korn H, et al, editor (2019) Biodiversity and health in the face of climate change. Cham (Switzerland): Springer.
2) G. Erdtman (1960) Svensk Botanisk Tidskrift, 54(4), 561-564.
3) A. Florenzano, A.M. Mercuri, A. Pederzoli, P. Torri, G. Bosi, L. Olmi, R. Rinaldi, Bandini M. Mazzanti (2012) Geoarchaeology, 27, 34–47. https://doi.org/10.1002/gea.21390.

## 3.6 = Palynology on reconstructing the long-term dynamics of plant biodiversity: Insights from nature-value areas in Italy – Preliminary analysis from Pollino National Park (southern Italy)

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The Pollino National Park (PZ, MT, CS provinces) is the largest protected area in Italy and was designated as a UNESCO World Heritage Site in 2015. It harbors many endemic species, Quaternary refuges of temperate trees, and old-growth forests. An example is the remaining mixed beech-fir forest, a priority habitat for the biodiversity conservation in Europe. Old-growth forests – with their structural heterogeneity, microhabitats, and ecological continuity - are important for biodiversity and carbon storage, making them of great conservation interest.

In this study, we present the preliminary palynological analysis of the STAPE site ("Stagno del Pesce", Francavilla in Sinni, PZ) near the Rubbio Nature Reserve, established in 1972 and currently part of the Natura 2000 Network for the presence of the Apennine beech-fir mixed forest (code: IT9210300). The STAPE site is located within a pure beech forest. The aim is to reconstruct and monitor the long-term dynamics of forest biodiversity in an area of significant conservation importance.

We analyzed 15 samples taken from two sediment cores (2B and 3B), with one core consisting of two sections (2B-1L and 2B-2L), reaching a depth of 167 cm (dated 299 ± 33 uncal. $C^{14}$ BP). The preliminary pollen diagrams show high forest cover, with some curve oscillations. The forest community is dominated by fir (*Abies* sp.), beech (*Fagus sylvatica*), and deciduous oaks (deciduous *Quercus*). Fir and beech exhibit complementary dynamics along the entire sequence. Fir prevails in deeper levels and shows a decreasing trend towards the more superficial levels where beech dominates. Pine (*Pinus* sp.), mixed oakwood taxa (such as *Acer campestre* type, *Carpinus betulus*, *Fraxinus excelsior* type), Mediterranean plants (*Olea*, *Quercus ilex* type, *Fraxinus ornus* type) and hygrophilous trees (*Alnus* and *Salix*) represent minor components of forest cover. The herbaceous vegetation consists of hygrophilous herbs (Cyperaceae undiff., *Scirpus*, *Sparganium emersum* type) indicating the presence of wet environments and by wild Poaceae. Cereals, other anthropogenic pollen indicators (API) and pastoral pollen indicators (LPPI) are found in lower percentages but more abundant in certain levels.

These preliminary results highlight the complexity of forest dynamics and the historical presence of fir. Silver fir is a species highly sensitive to direct anthropic pressures (e.g., grazing, fires, forest exploitation). Further investigations are required to deepen these preliminary results, trying to reach a more specific pollen identification level and to explore relationships of plants with anthropic and/or climatic factors.

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## 3.6 = Palaeoenvironment and land-use at the UNESCO archaeological site of Palù di Livenza (Pordenone): a palynological perspective

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Palynological studies in wet environments can be particularly informative of past biodiversity because pollen is usually very well preserved and abundant. Palù di Livenza is a Neolithic pile-dwelling site, inscribed in the UNESCO World Heritage List since 2011. Investigations coordinated by Soprintendenza ABAP-FVG brought to light an impressive amount of archaeological and palaeobotanical data for the knowledge of the life in wetlands during the Neolithic.

In this study, we present the palynological analyses performed on three sequences: two were taken from on-site trenches, and one was cored off-site. The on-site records (PaluON1 and PaluON2 for a total of 40 samples) were vertically collected in Sector 3, while the off-site record (21 samples) was collected in the basin of the Livenza, 300 meters south of it, up to a depth of 6 m. The aim of the study is to reconstruct an image of the vegetation cover and its changes through time as well as deepen some aspects related to the land-use of the territory between c. 6350 and 5600 cal BP (c. 4400 and 3650 BC).

The results from the off-site core show high presence of arboreal pollen (AP), mainly belonging to taxa of the mixed oakwood and in lesser extent of the hygrophilous wood, indicating a forested environment and the presence of some wet areas around the site. From the centre of the sequence, the oakwood gradually decreases in favour of the hygrophilous taxa (both arboreal and non-arboreal taxa), indicating the establishment of a wetter environment. Cereals, other Anthropogenic Pollen Indicators (API, (1)) and Local Pastoral Pollen Indicators (LPPI, (2)) are present throughout the sequence with little but significant amount.

The results from one of the two on-site records (PaluON1, recently published (3)) shows high forestation rate in the pre-settlement phase indicating a forested environment, dominated by the mixed oakwood and the hygrophilous wood. During the archaeological phases, a decrease in the forest cover is highlighted in the pollen diagrams. In these phases, cereals and other API are high indicating the presence of cereal fields in the area. Moreover, an area of the settlement was devoted to food processing by Neolithic communities. In the layers of the sequence corresponding to the end and after the abandonment of the settlement, a new forestation occurs mainly dominated by the hygrophilous wood indicating the establishment of a wooded swamp at Palù.

Combining the information obtained from both the on-site sequences and the off-site core a detailed reconstruction of the palaeoenvironment at Palù during Neolithic emerges. Land-use was locally diversified and characterised by crop fields, and to a lesser extent by pasturelands.

This study is included in a PhD PON project (entrusted to J.Z.) aiming at reconstructing the biodiversity, the environment and the human/environment dynamics in Northern Italy from the Neolithic period to the end of the Bronze Age.

1) A.M. Mercuri, M. Mazzanti, A. Florenzano, M. C. Montecchi, E. Rattighieri, P. Torri (2013) Annali di Botanica, 3, 143-153.

2) F. Mazier, D. Galop, M.J. Gaillard, C. Rendu, C. Cugny, A. Legaz, O. Peyron, A. Buttler (2009) Holocene, 19(2), 171-188.

3) J. Zappa, N. Degasperi, M. Bassetti, A. Florenzano, P. Torri, G. Servera-Vives, A.M. Mercuri, R. Micheli (2023) Quaternary, in press.

## 3.7 = Habitats and endemic plants of the South-Western Alps: evolutionary and historical dynamics versus human activities on alpine plants with conservation priorities

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*Focus of the work*: In order to preserve the diversity of alpine flora, it is necessary to know its evolutionary history and particularly, how climate and man acted on the distribution of the most representative species. Through a phylo-geographical approach aimed at the study of population variability and genetic structure, past climatic factors and the impact of pastoral practices will be analyzed as main drivers of genetic variability and demographic history of the endemic species. Results of this study will help to better understand contingent evolutionary events and adaptation of the plants to a changing climate also to guide *in situ* and *ex situ* conservation.
*Expected results*: Its now well established that plants which currently live in the diversity hotspots of the Alps have survived major climatic upheavals of the Quaternary period. The series of glaciations first, and then ice-sheet retreat have shaped species distribution and their genetic assets through isolation in refuge areas, or migration and speciation in new habitats. However, the practice of pastoralism and land use have more recently conditioned prairie species through range expansions or regressions dating to the Bronze Age (ca. 5600 years ago), when these practices established in the Alps. The imprint of anthropogenic factors on the genetic structure of prairie communities is analyzed by comparing species and populations also in more conservative habitats than the prairie, characterized by a lower synanthropic character.
*Areas of intervention*: The herbaceous communities of the alliance *Avenion sempervirentis* and *Festuco scabriculmis-Potentilletum valderiae* which are almost typical dry prairies of the Maritime Alps will be studied as among the richest in endemism for this sector of the Alps. *Saxifragion lingulatae* with its typical endemic rupicolous communities will serve as a comparison habitat (Table 1).

Table 1

| **Species name** | **Phytosociological optimum** | **Endemisms [Geographic boundaries]** |
|---|---|---|
| All. ***Avenion sempervirentis*** Barbero 1968 | | |
| *Helictotrichon sempervirens* (Vill.) Pilg. | *Avenion sempervirentis* | SW Alpic [F, PIE, LIG]. |
| *Eryngium spinalba* Vill. | *Avenion sempervirentis* | SW Alpic [F, PIE, LIG]. |
| *Ononis cristata* Mill. subsp. *cristata* | *Avenion sempervirentis* | SW Alpic [PIE, LIG] |
| Ass. **Festuco scabriculmis-Potentilletum valderiae** Guinochet 1938 | | |
| *Festuca scabriculmis* (Hack.) K.Richt. | *Festucon variae* | SW Alpic [F, PIE, LIG, VDA] |
| *Veronica allionii* Vill. | *Caricetalia curvulae* | SW Alpic [F, PIE, VDA, LIG] |
| *Potentilla valderia* L. | *Festucion variae* | SW Alpic [F, PIE] |
| *Nigritella corneliana (Beauverd) Gölz & H.R.Reinhard* | *Seslerietalia variae* | *SW Alpic [F, PIE, LIG]* |
| All. **Saxifragion lingulatae** (Rioux & Quézel 1949) Loisel 1951 | | |
| *Micromeria marginata* (Sm.) Chater | *Saxifragion lingulatae* | SW Alpic [F, PIE, LIG] |
| *Helianthemum lunulatum* (All.) DC. | *Saxifragion lingulatae* | SW Alpic [F, PIE, LIG] |
| *Silene campanula* Pers. | *Saxifragion lingulatae* | SW Alpic [F, PIE, LIG] |
| All. **Arrhenatherion elatioris** Koch 1926 | | |
| *Campanula rhomboidalis* L. | *Campanulo rhomboidalis-Trisetenion flavescentis* | Alpic [F, PIE, VDA, LIG, LOM] |
| All. **Thlaspion rotundifolii** Jenny-Lips 1930 | | |
| *Campanula alpestris* All. | *Thlaspion rotundifolii* | SW Alpic (see also in Flora Europea) |

Acknowledgements
The authors acknowledge the support of NBFC to University of Torino, funded by the Italian Ministry of University and Research, PNRR, Missione 4 Componente 2, "Dalla ricerca all'impresa, Investimento 1.4, Project CN00000033.

## 3.7 = X-rays microCT as a prediction tool for seed germinability and viability: a case study with the Italian endemic *Hieracium australe* subsp. *australe*

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The evaluation of seed plant germination capacity is a fundamental part of many conservation actions, particularly in case of *ex situ* collections. Seed viability testing allows to estimate the proportion of live seeds in wild populations, *ex situ* living accessions (e.g., in botanic gardens), and seed samples and lots (e.g., seed banks and industries). However, concerning threatened species with no *ex situ* collections and extinct species, one and sometimes the only seeds that can be used for conservation actions can be found in natural history collections, especially herbaria (1, 2). The use of *herbaria* as a source of seeds poses several concerns, among which the specimen storage conditions that are not suitable for seed viability conservation over time, use of pesticides and freezing cycles that can alter DNA and tissues structures and/or seed dormancy, and limited number of mature, intact, and collectable seeds, especially in case of threatened and extinct species. Considering these and other constraints related to the use of such seeds for conservation purposes, non-disruptive and non-invasive techniques for seed viability and germinability assessment are needed.
Therefore, this study aims at assessing whether the use of X-ray microCT imaging on an herbarium sample of *Hieracium australe* Fr. subsp. *australe* (Italian endemic taxon related to the extinct *Hieracium tolstoii* Fen. & Zahn) seeds can be used as non-invasive techniques for seed viability and germinability prediction. In addition, a comparison of the predictive potential of qualitative traits using multinomial logistic regression and quantitative ones using linear discriminant analysis (LDA) on germination capacity is provided.
Twenty-nine seeds of *H. australe* subsp. *australe* were scanned with X-ray microCT, at 30 kV and 200 μA for 54 minutes, and qualitative codes were assigned to each seed (Fig. 1). After scanning, 3D images were reconstructed to characterize qualitatively and quantitatively, internal and external morphology of each seed (Fig. 2). Also, germination tests on the scanned seeds have been done to evaluate seed germinability. The prediction resulting from the qualitative analysis showed a good performance (81.8%) for the prediction of the viability, but a poor prediction of the general germination outcomes (51.9%). Results from the quantitative analysis based on 18 morphometric parameters allowed to accurately relate different morphological seed traits with the germination outcomes (at least 80% of seeds correctly classified).
In conclusion, X-ray microCT combined with 3D image analysis is a very promising, non-disruptive approach for both high-resolution seed phenotyping and seed quality evaluation, that can be further implemented and applied for the propagation of threatened and even extinct species.

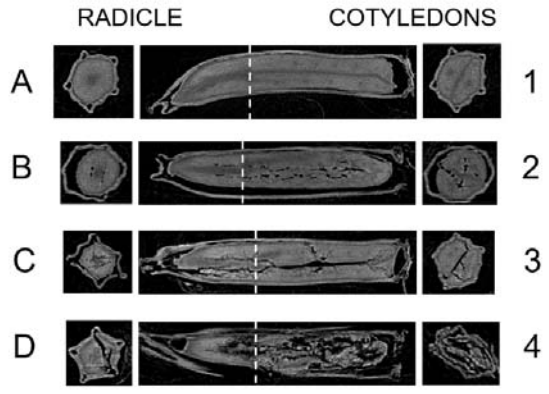


Fig. 1: Examples of micro-CT cross and longitudinal sections of *Hieracium* seeds and the corresponding assigned quality code.

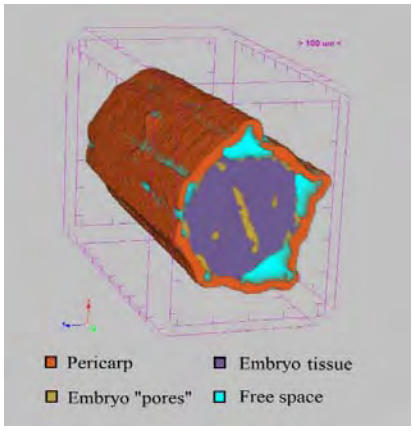


Fig. 2. Seed components segmented and separately analyzed to determine the morphometric parameters.

1) T. Abeli, S. Dalrymple, S. Godefroid, A. Mondoni, J.V. Müller, G. Rossi, S. Orsenigo (2020) Conservation Biology, 34(2), 303-313.
2) G. Albani Rocchetti, A. Carta, A. Mondoni, S. Godefroid, C.C. Davis, G. Caneva, ... T. Abeli (2022) Nature Plants, 8(12), 1385-1393.

## 3.7 = Conservation of the endangered endemic species of frankincense and myrrh tree species (Burseraceae) in Socotra Archipelago (Yemen)

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The Socotra Archipelago (Yemen) is a globally important biodiversity hotspot, with a significant proportion of endemic species that have evolved to survive in an arid subtropical environment, and was inscribed as a World Heritage Site by UNESCO in 2008. The terrestrial ecosystems of Socotra face several threats, including climate change, overgrazing and soil degradation. Socotra Island hosts four endemic species of the genus *Commiphora* (Myrrh trees, 1, Fig. 1) and eleven endemic species of the genus *Boswellia* (Frankincense trees, 2, Fig. 2). A rapid and significant decline of these species has been recorded (3), however a complete knowledge of the taxonomical and ecological features, and conservation status is still lacking preventing the identification of effective conservation and restoration strategies.
In a project founded by the Franklinia foundation, according to the indications provided by the Global Strategy for Plant Conservation a multicriteria approach was implemented involving local communities and scientists as a capacity building process. Activities included:

- A systematic sampling of the current distribution and population structure of the target species using field survey supported by drones to sample the most inaccessible sites.
- Assessment of the main threats.
- Collection of leaves according to a standardized protocol to conduct phylogenetic and population genetic analyses.
- Collection of resin to assess their chemical features for their sustainable commercial exploitation.
- Re-assessment of the conservation status according to the IUCN Red List also including the impact of climate change.
- Identification and implementation of conservation and restoration activities.

Results highlighted a significant worsening of the conservation status of all species due to the combined effect of overgrazing and the increasing frequency and intensity of extreme climatic events. The establishment of a network of plant nurseries is ongoing where seeds are collected from local populations and stored. Seedlings are replanted in fenced areas or protected by individual protection systems. Despite the positive outcomes of the project, an upscaled conservation and ecological restoration effort is required to prevent a rapid extinction of these unique and valuable species.

Fig. 1. *Commphira ornifolia*

Fig. 2. *Boswellia elongata*

1) D. La Montagna, F. Attorre, S. Hamdiah, P. Maděra, L. Malatesta, P. Vahalík, K. Van Damme, M. De Sanctis (2023) Front. For. Glob. Change, DOI: 10.3389/ffgc.2023.1183858
2) S. Hamdiah, L. Karas, K. Houšková, K. Van Damme, F. Attorre, P. Vahalík, H. Habrová, S. Lvončík, K. Eler, P. Maděra (2022) Plants, 11 (11), art. no. 1418.
3) F. Attorre, K.Van Damme (2020) Rendiconti Lincei, 31(3), 563 – 569.

## 3.7 = Quest for glory: how the Mediterranean monospecific endemic *Petagnaea gussonei* is fighting in the face of seed dormancy, climate changes and habitat fragmentation

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The Mediterranean region is a global hotspot of plant biodiversity, with ca. 30,000 taxa distributed across ten regional biodiversity hotspots (e.g., large islands), within which further smaller ones can be identified (e.g., local mountain chains). Sicily, the largest Mediterranean island with a complex geo-morphology, hosts over 10% of Mediterranean plants, of which an extraordinary number of endemic species, most notably range-restricted endemics. The relict species of the ancient Tertiary flora include *Petagnaea gussonei* (Spreng.) Rauschert (Fig. 1, 2, 3), an endemic monospecific plant that is heroically surviving despite its seed dormancy and its narrow geographical range, which is not only affected by dramatic climate changes but also by significant soil-use alteration. This study, in particular, investigated the germination capacity of *P. gussonei*, and the multi-temporal trends of soil-use, climate and desertification across the scattered range of *P. gussonei*. The final germination percentage (FGP) showed low values between 14 and 32%, the latter obtained with $GA_3$ and agar at 10 °C (Fig. 4). In the period 1931–2020, the average temperature increased by 0.5 °C, from 15.4 to 15.9 °C, in line with the projected climate changes throughout the 21st century across the Mediterranean region. These rising temperatures in the study area will further increase the dormancy of *P. gussonei*. The analysis of rainfall values showed that extreme events grew considerably in the period 1991–2020. The distributional area of *P. gussonei* is also affected by increasing desertification, which currently impacts 47.3% of the whole species range. The temporal changes of CORINE Land Cover classes created also a complex impacting mosaic where c. 40% are agricultural areas. The effective conservation of *P. gussonei* should be multilateral by relying on germplasm banks, improving landscape connectivity and vegetation cover, and promoting climate policies. This investigation was performed within the PON Project (Programma Operativo Nazionale), Research and Innovation (funded by the Italian Ministry of University and Research). The main aim of the project is to improve the conservation status of plant species of EU importance (Annexes II-IV of Directive 92/43/EEC) in Sicily (Italy). This project plays a crucial role in implementing conservation strategies aimed at reducing extinction risk.

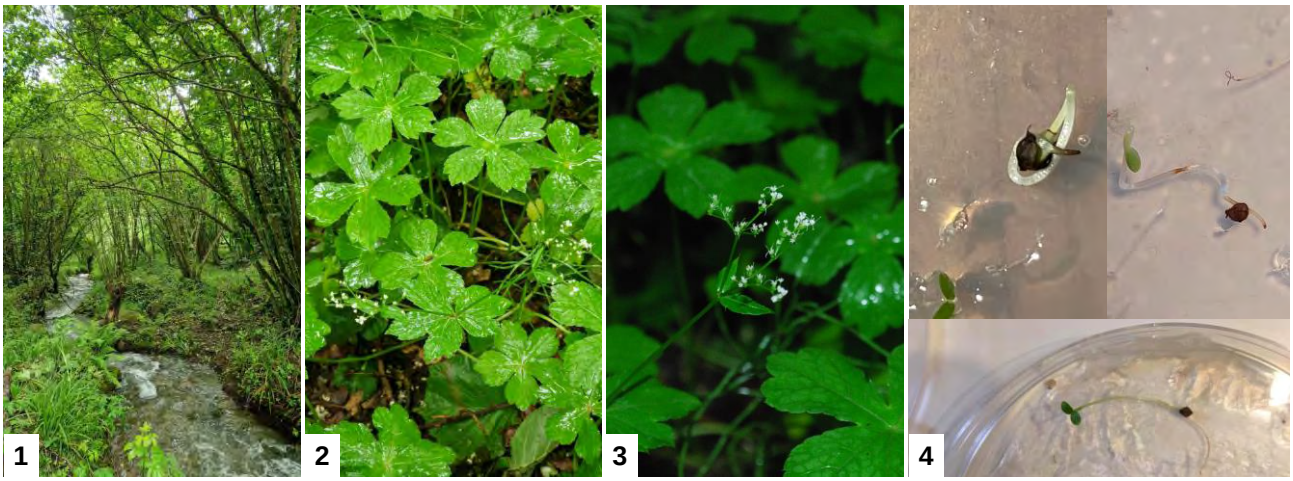


Fig. 1. Habitat of *Petagnaea gussonei*. Fig. 2. habit of *Petagnaea gussonei*. Fig. 3. inflorescences of *Petagnaea gussonei*. Fig. 4. germinated seeds of *Petagnaea gussonei*.

## 3.7 = Will the European Natura 2000 network of protected areas protect the endemic flora of the south-western Alps from climate change?

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[1]Department for the Earth, Environment and Life Sciences, University of Genova, Corso Europa 26, 16132 Genova, Italy

European Natura 2000 network of protected areas (PAs) is globally recognized as playing a key role for *in situ* conservation of species. Despite a high potential for biodiversity conservation, PAs may not mitigate the current biodiversity loss because they do not consider climate change impact (1). In fact, because PAs have static geographical boundaries, they may hardly keep pace with dynamics in species distribution caused by the climate change, which was not considered when they were designed. Therefore, in order to improve the assessment and realisation of the conservation potential of the Natura 2000 network, the use of modelling approaches to assess the effect of future climate is necessary (2). In this study, we used species distribution modelling of 85 plant taxa endemic or subendemic to Southwestern Alps to evaluate the effectiveness of PAs network in protecting endemics under future climate change scenarios. In the SW Alps the Natura 2000 network of PAs is extensive both in number and in coverage area, covering roughly the 30% of the area (Fig. 1). PAs will harbour more expected distribution range of taxa in the future than today, probably because they mainly occur in high altitude areas rich in endemics that have been climatically stable areas in the past and that will likely remain relatively climatically buffered in the future (Fig. 2). Moreover, PAs are distributed to poorly cover expected range of endemics at low and middle elevation, which are threatened by urbanization and climate change (Fig. 2). However, low-altitude populations may be genetically and morphologically differentiated from the rest of populations (3) and therefore worthy of protection. We recommend additional protection at low and middle elevation, considering the future climatic suitability of species. Furthermore, it must be considered that ensuring adequate representation of species within PAs is only a first step towards an effective network of PAs. Without effective management of protected sites, representativeness is useless.

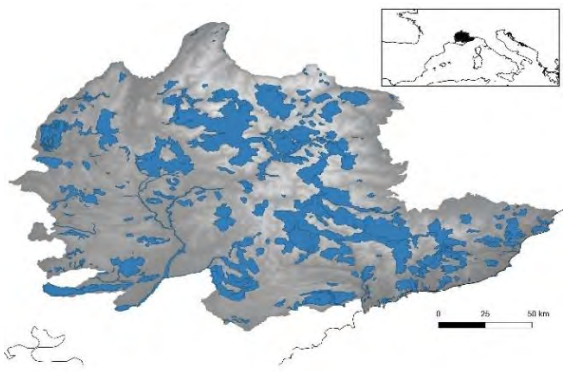


Fig. 1. The protected areas in the SW Alps.

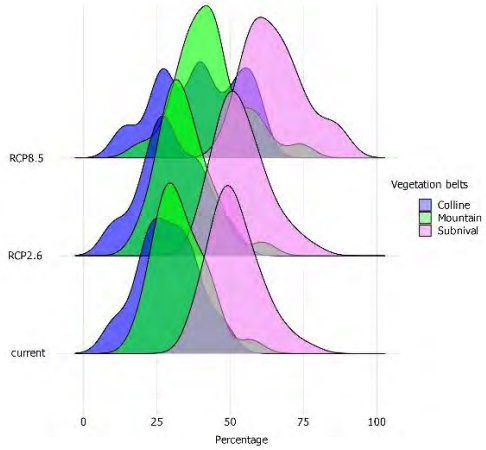


Fig. 2. Percentage of expected distributional range covered by PAs according to their vegetation belt.

1) P.R. Elsen, W.B. Monahan, E.R. Dougherty, A.M. Merenlender (2020). Sci. Adv. 6:eaay0814.
2) E.H. Orlikowska, J.M. Roberge, M. Blicharska, G. Mikusiński (2016) Biol. Conserv. 200, 216-227.
3) G. Casazza, C. Macrì, D. Dagnino, M. Juin, L. Minuto, J.D. Thompson, A. Baumel, F. Médail (2021) PeerJ 9:e11039.

## 3.7 = A survey of *ex situ* conservation of Italian endemics in the *Botanic Gardens Conservations International* (BGCI) network

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There are about 3,000 botanic gardens worldwide, approximately hosting 6 million accessions of living plants, corresponding to ca. 80,000 taxa. Among them, 1,202 institutions actively contribute to 'PlantSearch' (1), a global database implemented by the *Botanic Garden Conservation International* (hereafter BGCI), aimed to promote data sharing about *ex situ* conservation of living plant, seed, and tissue collections. We explored this database to assess the *ex situ* conservation status of Italian endemic taxa within the BGCI network. Italy is an endemic-rich country, hosting 1,475 taxa endemic to this area, spanning ca. 300,000 $km^2$ (2). For querying the database, we used both the accepted names according to the *Portal to the flora of Italy* (https://dryades.units.it/floritaly/index.php) and their synonyms (3); moreover, we did not consider cultivars.
We found that only 492 taxa (33%) are represented in *ex situ* collections. Concerning taxa subjected to *ex situ* conservation actions, we found a severely right-skewed distribution (mean = 5.3, median = 2.0, mode = 1.0). The taxon showing the highest *ex situ* representation is *Cerastium tomentosum* L. (Caryophyllaceae) with 112 records. From a taxonomical point of view, the year of the description of the taxon is negatively correlated with the number of *ex situ* conservation sites worldwide (Rho = -0.4, $p < 0.0001$). Focusing exclusively on the *ex situ* managed taxa, those at subspecific rank are numerically less represented than those at specific rank (Kruskal-Wallis Test, $p < 0.001$). From a conservation point of view, 121 taxa among those represented in *ex situ* collections are included in the IUCN Red List of Threatened Species, as: Data Deficient (10 taxa), Least Concern (39), Near Threatened (14), Vulnerable (18), Endangered (15), and Critically Endangered (25). No significant correlation was found between the taxon risk category and the number of *ex situ* sites. Focusing on Italian endemic taxa restricted to single administrative regions, we found the following ratios, expressing the number of regional endemics managed through *ex situ* conservation over the total number of regional endemics: Aosta Valley (0/6), Piedmont (3/26), Lombardy (7/21), Trentino-Alto Adige (0/21), Veneto (5/23), Friuli Venezia Giulia (5/15), Liguria (5/9), Emilia Romagna (1/8), Tuscany (30/72), Marche (1/11), Umbria (0/1), Lazio (1/13), Abruzzo (7/48), Molise (0/1), Apulia (10/40), Campania (3/25), Basilicata (0/10), Calabria (12/63), Sicily (82/292), and Sardinia (106/253). An assessment trough the PlantSearch database is likely affected by several biases, due to (a) taxonomic and nomenclatural issues, (b) outdated records, and (c) institutions not present in the database, but actually conserving *ex situ* the target taxa. Despite this, we can argue that these estimates are a proxy of the state of the art of the conservation of Italian endemics in botanic gardens. These results show that a significant fraction of these species is unrepresented in *ex situ* collections, determining concrete extinction risks for Italian endemics. A collective effort, primarily conducted by the Italian community of botanic gardens, should be implemented to foster the *ex situ* conservation of these species.

1) BGCI (2023). PlantSearch online database. Botanic Gardens Conservation International. Richmond, U.K. Available at https://tools.bgci.org/plant_search.php
2) L. Peruzzi, F. Conti, F. Bartolucci (2014) Phytotaxa, 168, 1-75.
3) L. Peruzzi, G. Domina, F. Bartolucci, G. Galasso et al. (2015) Phytotaxa, 196, 1-217.

## 3.7 = What community completeness can tell about the conservation status of ecosystems: a test on vascular plants in *Fagus sylvatica* forests

Emanuele Fanfarillo[1,2], Mariasole Calbi[3], Leopoldo de Simone[1], Tiberio Fiaschi[1], Bruno Foggi[3], Antonio Gabellini[1], Matilde Gennai[3], Simona Maccherini[1,2], Emilia Pafumi[1], Daniele Viciani[2,3], Giulio Zangari[4], Claudia Angiolini[1,2]

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Beech (*Fagus sylvatica* L.) forests are among the habitats with the highest naturalness in Europe (1). They are often considered as species-poor ecosystems since beech is highly competitive especially for light, but examples of species-rich beech forests are also well-documented. It is unclear if the lack of species in many beech forests is due to natural drivers or to anthropic management. In this study we investigated the patterns of plant community completeness (2) in the beech forests of Tuscany, central Italy. Floristic and structural attributes were surveyed in 155 circular plots of 8 m radius in 2020-2021. The regional species pool of beech forests was retrieved from about 1,100 vegetation relevés available in the literature. We built a series of Structural Equation Models based on Generalized Linear Modelling to assess the direct and indirect effects of anthropogenic and environmental variables on beech forests' community completeness. We tested if such effects differed according to forest types on the groups resulting from a modified TWINSPAN cluster analysis: 1) species-rich, low-elevation and basiphilous beech forests characterized by *Cardamine bulbifera, Hedera helix, Mercurialis perennis*; 2) species-poor acidophilous beech forests characterized by *Festuca heterophylla, Luzula pedemontana, Veronica officinalis*; 3) species-poor microthermal beech forests characterized by *Oxalis acetosella, Rubus idaeus, Dryopteris dilatata*. For the whole dataset, the lack of species at a given site was due to both anthropic and natural factors (Fig. 1): 1) silvicultural management (low DBH, indicating coppicing or recent cuts in high forests) that disadvantages understorey species; 2) low slopes that naturally promote litter accumulation suppressing understory species; 3) increasing elevation, with a natural loss of species. The selected predictors had different effects on the community completeness of the three forest types, with the main causes of species absence being high elevations in type 1, none of the selected predictors in type 2, and silvicultural management and low slopes in type 3. We conclude that community completeness can be used as an indicator of the conservation status of beech forests, since testing the role of different drivers in relation to forest type allows distinguishing natural lacks of species from those induced by human influences.

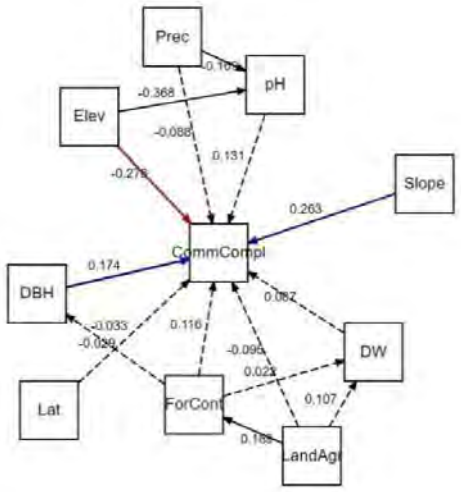


Fig. 1. Structural equation model on the community completeness of Tuscan beech forests. Continuous lines = statistically significant effects. CommCompl = community completeness; Prec = precipitation; Elev = elevation; DBH = mean Diameter at Breast Height; DW = total deadwood; ForCont = forest continuity; Lat = latitude; Land Agr = percentage of agricultural land use in the landscape. Blue = positive effect; red = negative effect.

1) G. Abbate, G. Pirone, G. Ciaschetti, S. Bonacquisti, E. Giovi, D. Luzzi, E. Scassellati (2003) Fitosociologia, 40(1), 97-108.
2) M. Pärtel, R. Szava-Kovats, M. Zobel (2013) Folia Geobot., 48, 307-317.

## 3.7 = The "Angelo Rambelli" Botanical Garden's wild orchids: a novel monitoring strategy

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The *Orchidaceae* family is the second largest family of flowering plants and includes several threatened organisms; 519 orchid species were listed on the IUCN Red List. They are subjected to several protection measures on a local, national, and international level (1). In 2004, the "Angelo Rambelli" Botanical Garden began taking conservation and protection measures for native orchids found on Tuscia's territory in collaboration with the Tuscia Germplasm Bank and the local G.I.R.O.S. section (2). As part of the project various orchid species were selected for *ex situ* conservation. Globally, 25 *taxa* were introduced into the Botanical Garden and placed in different habitats (travertine outcrops, undergrowth, meadows, etc.) according to their ecological needs (3). The plants transferred into the Botanical Garden were taken from threatened natural populations in the Tuscia area. In addition, there were seven species of spontaneous orchids, four of which belonged to the *Ophrys* genus, in the northern part of the Botanical Garden, which was characterized by travertine. In 2014, the native orchid collection hosted about 30 *taxa* belonging to 12 genera. The constant monitoring showed a general evolution of the populations with the loss of some species and the expansion of others. Considering the observed dynamics, it was decided to develop a new management tool to track orchid populations in the Botanical Garden. This new system realized in QGis 3.26 software, was elaborated using various layers. After delimiting the border of the Botanical Garden by obtaining the margins from the cadastral sheets, two shapefile layers were created: a polygonal shapefile layer covering the entire study area (4,8 hectares) with a grid composed of 153 square cells of 400 m² area, to survey the individuals of the most common and widespread species (*Ophrys passionis* subsp. *garganica* E.Nelson ex H.Baumann & R.Lorenz); a points shapefile layer set up to record all individuals belonging to other orchid species. As ground truth were used two layers: a first one containing proper up-to-date satellite images and the other, produced in previous years by the Botanical Garden's staff, enclosing the position relative to all the trees from the Botanical Garden collections. Then, by means of the QField app, data from new observations were recorded during periodic field trips. Lastly, by post-processing, it was also possible to provide immediate visual data. During the period of the first census with this new method (October 2022 - May 2023) 12 species were recorded. They are included in 4 different genera: *Anacamptis* (4 species), *Barlia* (1 species), *Neotinea* (1 species), *Ophrys* (4 species), *Orchis* (1 species), and *Spiranthes* (1 species). The most common one, *O. passionis* subsp. *garganica*, showed a substantial expansion compared to past years. Even other introduced species such as *Anacamptis pyramidalis* (L.) Rich., *Ophrys bombyliflora* Link, *Orchis italica* Poir. and *Spiranthes spiralis* (L.) Chevall., showed an expansion trend with the spontaneous colonization of some new areas during the years. On the other hand, 19 of the introduced species are no longer present and this must be due to a combination of factors, including soil conditions, transplant stress, and the presence of animals that are harmful to orchids. The newly adopted monitoring system should now make it possible to identify population dynamics with greater accuracy than in the past, offering a useful resource for making decisions about future management or new introduction policies.

1) J. Wraith, C. Pickering (2018) Ambio, 47, 307–317.
2) S. Magrini, M. Fonck, L. Zucconi (2014) Esperienze di conservazione delle orchidee. Orto Botanico della Tuscia, Viterbo.
3) S. Magrini, S. Buono, M. De Vitis, G. Haile, M. Rempicci, E. Gransinigh, E. Scarici, M. Fonck (2014) J. Eur. Orch., 46(1), 103-114.

## 3.7 = An assessment of the extinction risk for *Santolina decumbens* subsp. *tisoniana* (Asteraceae), a recently described taxon endemic to France

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*Santolina decumbens* Mill. (Asteraceae, Anthemideae), commonly known as "creeping hoary lavender-cotton", is endemic to Provence-Alpes-Côte d'Azur (southern France). In this region, this species grows mostly on limestone where it forms garrigues from the sea level up to 1200 m a.s.l. (1). As most species within *Santolina*, it is adapted growing in extreme conditions of aridity and sun exposure. In a recent taxonomic study (2), three allopatric subspecies were recognized. *Santolina decumbens* subsp. *decumbens*, the most white-tomentose, occurs from Marseille eastwards to Toulon and northwards up to Aix-en-Provence on Montagne de Sainte-Victoire; *S. decumbens* subsp. *diversifolia* (Jord. & Fourr.) Giacò & Peruzzi, less tomentose and showing longer stems and leaves, is endemic to the department of Alpes-de-Haute-Provence; *S. decumbens* subsp. *tisoniana* Giacò & Peruzzi, less tomentose and showing shorter stems and leaves, is endemic to a restricted area in the department of Bouches-du-Rhône. As concerns the latter subspecies, based on field observations, the authors (2) claimed the urgent need of an extinction risk assessment following the IUCN criteria and protocol (3). The distribution range of this subspecies spans in an area that is less than 25 $km^2$. In addition, most of the individuals were observed occurring in a very anthropized area subjected to the expansion of suburbs and industrial factories. Moreover, climate change was listed as additional threat, since it would cause the extremization of already extreme environmental conditions. Due to the restricted range and the habitat modifications, *S. decumbens* subsp. *tisoniana* qualifies as a critically endangered (CR: B1ab(iii) 2ab(iii)) taxon. Both *ex situ* (e.g., seed storage in seed banks and cultivation in botanic gardens) and *in situ* conservation projects are needed to ensure the survival of this subspecies.

1) J.-M. Tison, B. de Foucault (2014) Flora Gallica–Flore de France. Mèze: Biotope Éditons.
2) A. Giacò, L. Varaldo, G. Casazza, D. De Luca, P. Caputo, M. Sarigu, G. Bacchetta, L. Sáez, L. Peruzzi (2022) J Syst Evol, https://doi.org/10.1111/jse.12925
3) IUCN (2022). The IUCN Red List of Threatened Species. Version 2022-2. https://www.iucnredlist.org. Accessed on 25 May 2023.

## 3.7 = Monitoring plants in Tuscany: the Nat.Ne.T project

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Monitoring is vital for the *in situ* conservation of protected plant species (1). The "Habitats" Directive (92/43/EEC) mandates EU member states to monitor Community Interest habitats and species of and assess their conservation status. In Italy, the Ministry for the Environment oversees this implementation and requests monitoring data from regional administrations (2). The Nat.Ne.T - Natura 2000 Network Tuscany is a joint project of Regione Toscana and Tuscan universities (Florence, Pisa, and Siena) which aims to evaluate the conservation status of Tuscan habitats and species of Community Interest through a multi-year monitoring campaign.
The PLANTSEED Lab at the University of Pisa is responsible for monitoring plant species. Since 2018, species of Annexes II, IV, and V have been monitored in various locations, some over multiple years (Table 1). The monitoring campaign is scheduled to continue until 2024, aiming for comprehensive taxonomic and geographic coverage. Field excursion destinations were selected using the online database Wikiplantbase #Toscana and scheduled at flowering times of target species.
Surveyors collected station data, population size and identified pressures and threats. These efforts have provided valuable insights into the distribution and status of target species, enabling updates to Standard Data Forms. The monitoring data also support related projects on selected target species, including the Italian endemics *Primula apennina* and *Aquilegia bertolonii*. These data improve *in situ* conservation and supports actions for *ex situ* conservation.

Table 1. Species included in the monitoring campaign and year of monitoring.

| Species | Annex | Year | Species | Annex | Year |
|---|---|---|---|---|---|
| *Buxbaumia viridis* | II | 2022, 2024 | *Crocus ilvensis* | IV | 2018, 2024 |
| *Leucobryum glaucum* | V | 2024 | *Eleocharis carniolica* | II, IV | 2020, 2023 |
| *Mannia triandra* | II | 2024 | *Galanthus nivalis* | V | 2018 |
| *Orthotrichum rogeri* | II | 2022, 2024 | *Galanthus reginae-olgae* | V | 2018 |
| *Sphagnum* spp. | V | 2018, 2024 | *Gentiana lutea* | V | 2023 |
| *Lycopodium* spp. | V | 2018, 2023 | *Gladiolus palustris* | II, IV | 2018, 2021 |
| *Marsilea quadrifolia* | II, IV | 2018, 2023 | *Hibiscus pentacarpos* | II, IV | 2018 |
| *Trichomanes speciosum* | II, IV | 2018, 2023 | *Himantoglossum adriaticum* | II, IV | 2021, 2023 |
| *Aldrovanda vesiculosa* | II, IV | 2018 | *Ionopsidium savianum* | II, IV | 2018, 2023 |
| *Aquilegia bertolonii* | II, IV | 2021, 2023 | *Liparis loeselii* | II, IV | 2018 |
| *Aquilegia lucensis* | IV | 2021, 2023 | *Primula apennina* | II*, IV | 2018, 2023 |
| *Athamanta cortiana* | II, IV | 2018 | *Ruscus aculeatus* | V | 2018 |
| *Caldesia parnassifolia* | II, IV | 2018 | *Spiranthes aestivalis* | IV | 2021, 2024 |
| *Crocus etruscus* | IV | 2018, 2024 | | | |

1) V.H. Heywood (2019) Plant Diversity, 41(2), 36-49.
2) S. Ercole et al. (2016) ISPRA, Serie Manuali e linee guida 140/2016.

### 3.7 = The new "E. Dioli" Botanical Garden in Valmalenco (SO, Italy): from ethnobotany a means to the conservation and promotion of autochthonous plant species

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Within the framework of the European Interreg Italy-Switzerland B-ICE project (2019-2022) and the current B-ICE GEMME project (2022-2023), following an ethnobotanical investigation that involved 401 inhabitants of Valmalenco (SO, Italy), the conceptualization, designing, and realization of two Botanical Gardens on the territory have been taking place. Specifically, the "E. Dioli" Botanical Garden in Sant'Antonio di Caspoggio (1,300 m a.s.l.) and its branch, the Didactic Botanical Garden in Caspoggio (1,098 m a.s.l.) were designed with the main purpose of preserving the traditional knowledge concerning autochthonous plant species and promoting new strategies, in accordance with the primary objectives of the projects, for the sustainable development of the area. As a matter of fact, as Alpine regions have been suffering more and more due to the ongoing climate crisis, searching for valuable alternatives to winter tourism has become pivotal. Cultural tourism may represent such an alternative and the two Botanical Gardens, so closely intertwined with the traditions of the valley, may be the keystone of this new development.
Focusing on the "E. Dioli" Botanical Garden, special attention was paid to the accessibility of the spaces, the sustainability of the materials used, and the usability of the layout by different types of audiences.
The Garden is named after Erminio Dioli, architect and artist born in Caspoggio at the end of the 19th century, with the purpose of creating a dedicated cultural circuit along with the homonymous museum in Caspoggio on the life and works of the artist.
The Garden was designed to host 70 selected plant species arranged in 8 flowerbeds, each for every traditional field of use. With the involvement of experts, the total area extension, maximum height reached by the plants, potential incompatibility among the species, special needs in terms of soil, watering, and pedo-climatic characteristics, level of protection, and aesthetics were taken into consideration.
New illustrative panels and *ad hoc* high-level labels were created for each plant species with the following information: botanical family, scientific name, common and vernacular name, botanical characters, *habitat* and altitudinal zone, protection, balsamic period, part of the plant used, active compounds, and traditional uses identified during the field investigation. With a view to inclusiveness, the interpretative apparatuses also contain information in Braille and a QR code for accessing to the content in English.
Within the context of the development of local resources and as part of a Citizen Science framework, a three-part course for local guides and volunteers was planned, starting from November 2022; it took place with the aim of selecting and training people in the management of the yearly summer opening. Jointly with the selected guides and other experts, a schedule of weekly activities, laboratories, guides, and tours was developed for both children and adults, with special attention to people with disabilities. Additionally, in association with professional illustrators, booklets for children, leaflets, bookmarks, and gadgets were created in order to help the self-support of the Garden, which will be inaugurated in July 2023. Finally, the website is currently under development.
In the framework of a cyclic ethnobotanical investigation, the "E. Dioli" Botanical Garden represents a precious and effective means to abide by the Open Science features of the project, as an area suspended in time and space, where past and present traditions meet through the selection of plant species.

## 3.7 = The Importance of Freshwater ecosystems for Lichen Diversity in Central Italy

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Riparian vegetation is an integral part of freshwater ecosystems. Riparian plant communities along streams and rivers are composed by distinctive species, have also distinctive structure and regeneration processes, appearing as a unique biological community with important ecological functions in maintaining biodiversity. Main aim of this study is to increase in lichen basic knowledge regarding riparian forest habitats (according to Habitats Directive), often threatened by agricultural activities, penetration of alien species and urbanization which cause the reduction and isolation of native populations, the decline of biodiversity, the increase of local extinction of native species and the expansion of generalist species (1). The study was carried out in 18 sites localized along the river Nera (Umbria), Mignone, Fiora, Olpeta, Aniene and Amaseno (Lazio), Volturno (Molise), and Mingardo and Fasanella (Campania) (Fig. 1). Epiphytic lichen flora was recorded on *Alnus* spp., *Populus* spp., and *Salix* spp. characterizing the riparian habitats 3280 "Constantly flowing Mediterranean rivers with Paspalo-Agrostidion species and hanging curtains of *Salix* and *Populus alba*", 91E0* "Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (Alno-Padion, Alnion incanae, Salicion albae)", and 92A0 "*Salix alba* and *Populus alba* galleries". 79 species were recorded: 6 in 3280 (1 exclusive), 30 in 91E0* (18 exclusive), and 60 in 92A0 (43 exclusive) (Fig. 2). Among these species: 3 (*Bacidia absistens*, *Porina oxneri* and *Traponora varians*) are assessed as vulnerable (VU) in the last Italian red list of epiphytic lichens, 5 (*Arthonia vinosa*, *Arthopyrenia salicis*, *Diarthonis spadicea*, *Fellhanera bouteillei* and *Thelenella modesta*) near threatened (NT) (2); 7 are indicator of ancient, undisturbed forests (the threatened *A. vinosa*, *F. bouteillei*, and *P. oxneri*, and also *Acrocordia cavata*, *Bacidia fraxinea*, *B. laurocerasi*, *Diarthonis spadicea*), 19 are species occurring in natural or semi-natural habitats (*Arthonia didyma*, *Arthopyrenia salicis*, *Bacidia absistens*, *B. arceutina*, *Bacidina phacodes*, *Collema furfuraceum*, *Coniocarpon cinnabarinum*, *Diromma dirinellum*, *Fuscopannaria ignobilis*, *F. mediterranea*, *Lecanora strobilina*, *Leptogium brebissonii*, *Mycobilimbia sphaeroides*, *Mycomicrothelia confusa*, *Pertusaria pustulata*, *Porina aenea*, *Pyrenula nitida*, *P. nitidella*, *Staurolemma omphalarioides*, *Thelenella modesta*, and the cited threatened species *T. varians* (3).
The bio-ecological characterization of the detected lichen flora highlights its close correlation with environmental parameters (e.g., light availability, humidity, etc.) strictly related to forest riparian habitats. Lichens are well known environmental bioindicators. Their distribution depends on both substrate (bark pH and texture)- and environment-related factors including atmospheric pollution (e.g., by agricultural practices). In these riparian habitats, changes in the floristic composition can be utilized as early warning signal to detect an environmental alteration. Therefore, the implementation of lichenological knowledge of these habitats, as well as having an intrinsic value, plays an important role in preventive conservation of biodiversity.

Fig. 1a-1b. Riparian vegetation of Mingardo and Fasanella.

Fig. 2. Epiphytic lichen colonization.

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1) C.A. Peres, T.A. Gardner, J. Barlow, J. Zuanon, F. Michalski, A.C. Lees, I.C.G. Vieira, F.M.S. Moreira, K.J. Feeley (2010) Biol. Conserv., 143, 2314-2327.
2) J. Nascimbene, P.L. Nimis, S. Ravera (2013) Plant Biosyst. 147, 898-904.
3) P.L. Nimis P.L., 2023. ITALIC - The Information System on Italian Lichens. Version 7.0. University of Trieste, Dept. of Biology, (https://dryades.units.it/italic), accessed on 2023, 05, 30.

## 3.8 = Dead but alive: increasing specimen collections at the Herbarium Horti Botanici Pisani

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Herbaria are the most relevant source of data related to plant diversity, so that they are still active in fostering expeditions, and take charge of the specimens collected, and collaborating with third-party botanists to accommodate their collections (1). The *Herbarium Horti Botanici Pisani* (the Herbarium of the Botanic Garden and Museum of the University of Pisa, PI according to Index Herbariorum) is fully committed to its role of documenting and preserving biodiversity data and, therefore, is partner in different national and international projects led by scholars of botany (2, 3). These partnerships imply the acquisition of new data through the collection of specimens, which are then available to the public. A further boost to the implementation of the quality and quantity of our collections has been given by the digitization activities started in 2017, thanks to the purchase of a Bookeye® 4 Professional planetary scanner and the access to private grants. As just one example, one of the richest collections preserved in our herbarium – the Herbarium of Michele Guadagno – was completely digitized after a four-years effort, during which more than 41,000 specimens originally collected from 1830 to 1929 were scanned and the metadata excerpted from the labels entered into the Virtual Herbaria JACQ online database (https://www.jacq.org/). However, new expeditions and new acquisitions have been made since the digitization activity started up, making a significant increase of the collections preserved in PI. Since 2017, our herbarium has acquired ca 7,015 specimens of vascular plants. The main geographical area involved is Tuscany (Central Italy), where ca 60% of the specimens were collected. This evidence underlines the close connection of our institution to the surrounding territory. However, ca. 9% is also represented by specimens collected in Calabria, a geographical area deeply investigated by two of the most active collectors present in this survey. The two main reasons for the observed increment are related to excursions organized by the Working Group of Floristics, Systematics, and Evolution of the Italian Botanical Society and research led by the PLANTSEED Lab of the Department of Biology of the University of Pisa. Other specimens come from studies devoted to the diversity and distribution of specific taxa, such as the genus *Alchemilla* in Tuscany, which also provided an impulse for the collection of plants and the preparation of *exsiccata*. More than half of the specimens were collected by staff members of the Botanic Garden and Museum (ca. 56%), but specimens donated by third-party collectors are relevant as well (ca. 35%). Another notable case are the specimens collected by students during their internships (9% of the specimens). This attests to the active role of our staff in floristic research and of our institution in providing support to the floristic research of external botanists, often amateurs, but also research from other departments. Indeed, the specimens collected for floristic purposes are the vast majority (ca. 77%), highlighting the importance of this kind of research to maintain herbaria as proactive structures. However, those used for systematic studies, carried on by the PLANTSEED Lab cited above, are considerable as well (ca. 21%). A special consideration must be given to the specimens collected by the mycologist Roberto Narducci, who donated more than 1,600 specimens of fungi, mostly from Tuscany, in the past few years. Accordingly, our herbarium is still today actively providing a relevant service for the scientific community.

1) J.M. Heberling (2022) Int. J. Pl. Sci., 183(2), 87-118.
2) L. Peruzzi, F. Roma-Marzio, L. Pinzani, G. Bedini (eds) (2019+). Wikiplantbase #Italia, http://bot.biologia.unipi.it/wpb/italia
3) WFO (2023): World Flora Online. Published on the Internet; http://www.worldfloraonline.org

## 3.8 = A multi-step study on *Phlomis fruticosa*: micromorphological and phytochemical insights

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A multi-level study was performed on the vegetative and reproductive organs of *Phlomis fruticosa* L. (Lamiaceae), cultivated at the Ghirardi Botanic Garden (Toscolano Maderno, Brescia, Italy). This work is part of a wider project entitled *Ghirardi Botanic Garden, factory of molecules…work in progress* (funded by Lombardy Region under the Call for the Enhancement of Museums Lr. 25/2016, year 2021), intended to preserve and enhance the plant heritage hosted at the Botanical Garden.

The multidisciplinary survey combined four sequential approaches of research: I) micromorphological and II) histochemical, to describe the structures responsible for the productivity of secondary metabolites, and to define the chemical nature of the secretory products, by means of Light Microscopy (LM) and Scanning Electron Microscopy (SEM); III) phytochemical, to characterize the composition of the essential oil (EO) obtained from the aerial parts at blooming by Clevenger-type hydrodistillation coupled with Gas Chromatography-Mass Spectrometry (GC-MS); IV) biological, through the combination of the morphological and phytochemical results with literature data referring to the potential biological activity of the most abundant EO compounds.

For the first time, the micromorphological survey on *P. fruticosa* combined observations both on the vegetative and reproductive organs, showing the presence of non-glandular and glandular *indumenta*. The non-glandular trichomes were multicellular dendritic or multicellular uniseriate, ubiquitous on the whole plant surfaces; the glandular ones were capitate belonging to three main morphotypes: branched stalked with a one-cell head (sporadic on leaves and flowers), with simple-short stalked with a one(two)-cell head (ubiquitous on the whole epidermises), simple-medium stalked with a four-cell head (exclusive of the reproductive organs). As an element of novelty, the histochemical study proved a complex chemical composition of the secretory materials produced by the branched and medium-simple capitate hairs, with dominant terpenes and minor polyphenolic and flavonoid fractions; the simple-short hairs were responsible for the secretion of mucopolysaccharides and acid polysaccharides. The analysis of EO composition revealed 37 different compounds, representing 100.0% of the total; oil yield was 0.043%. The profile was dominated by non-terpene derivatives (43.80%), followed by oxygenated monoterpenes (38.69%), with high relative percentages of santolina alcohol (40.93%) and 1,8-cineole (34.49%). Finally, based on literature data on EOs with santolina alcohol or 1,8-cineole as ones of the main compounds (1,2), antimicrobial, antioxidant, and anti-inflammatory properties were hypothesized for the EO analyzed here-in.

In the light of an *Open Science* policy, these results were merged in the realization of original iconographic and interpretative apparatuses on the target species at the Ghirardi Botanic Garden. In this way the results of the scientific research are made accessible to the visitors, giving the opportunity to discover the plant heritage under a novel perspective, starting from a macroscopic point of view towards its microscopic secrets.

1) J.J. Filippi, D.A. Lanfranchi, S. Prado, N. Baldovini, U.J. Meierhenrich (2006) J. Agric. Food Chem., 54(17), 6308-6313.
2) Z.M. Cai, J.Q. Peng, Y. Chen, L. Tao, Y.Y. Zhang, L.Y. Fu, Q.D. Long, X.C. Shen (2020) J. Asian Nat. Prod. Res., 23(10), 938-954.

## 3.8 = The coexistence of cultivated and wild flowers in the Botanical Garden of Siena and their Qualitative Pollination Networks

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The methods and means of pollination are just as important to insect pollinators as they are to plants. For insect pollinators, pollination is a way to find food and mates. For plants, pollination is essential for reproduction and the recombination of genetic characteristics. Insect pollinators and plants have co-evolved over time, and this co-evolution directed their development and cooperation. Entomophilous pollination therefore is particularly important for flowering plants and their insect pollen vectors. Botanical gardens are ideal for studying the coexistence of cultivated and wild flora (Fig 1.a and Fig 1.b). This provides an opportunity to identify which plant species benefit most from the presence of wild bees, and in particular, which species of wild bees.
In this study, a census of the apiform Apoidea present within the Botanical Garden of Siena was conducted between March and September 2023, taking into consideration a preliminary census conducted between April and July 2021, that had highlighted the presence of 131 morphospecies and an estimated 490 single interactions across 32 flowering plant species. The data collected includes both qualitative and quantitative information about interactions between flowering plants and their pollinators, as well as the capture of single Apoids for identification and cataloging.
The data collected will contribute to a comprehensive description of the Apoid community within the Botanical Garden of Siena, including the interactions between plant and pollinators. This information will help identify which plants should be prioritized for conservation efforts to protect their associated pollinating insects.

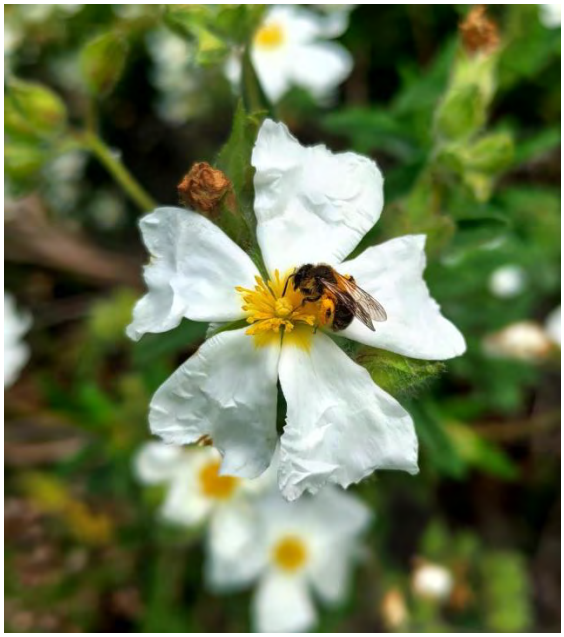

Fig 1. An *Andrena* ♀ sp. visiting a *Cistus monospeliensis* flower.

Fig 2. An *Andrena* ♂ sp. visiting a *Calendula* sp. flower.

### 3.8 = *Ycf1*, an uncommon DNA Barcode tested on *Cycas* collections from the Botanical Gardens of Padova and Palermo

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Botanical gardens typically identify and label their living plant collections using morphological observations. However, in some plant groups, morphology is often insufficient for accurate species identification. In these cases, specific DNA barcodes can be used to distinguish different species. The common barcodes for land plants, such as *matK* and *rbcL* (1), are inadequate to disentangle differences among various groups of plants, such as Cycads (2). Cycads are the second largest group of gymnosperms comprising three families, 10 accepted genera and 356 extant species, mostly belonging to the genus *Cycas*. Supplementary barcodes *trnH-psbA*, and ITS are not useful for DNA Barcoding in Cycads, due to difficulties in amplification and low substitution rate, respectively. In this work *ycf1* was tested, together with the core plant barcodes *matK* and *rbcL*, to study the variability among *Cycas* specimens stored in the Botanical Gardens of Padova and Palermo. Previous studies suggest *ycf1* as a potential DNA barcode of land plants due to its high variability (3). The specimens belonging to the genus *Cycas* from the two Botanical Gardens were collected and the DNA was extracted to allow the sequencing of various selected DNA barcodes. Phylogenetic, parsimony-informative sites and genetic variations analyses were performed using MEGA-10.1.8 and RAxML to compare the performances among the selected DNA barcodes. The effects of multiple gene regions on the resolution of species were also assessed by concatenation of different combination of DNA barcodes. In the genus *Cycas*, *ycf1* displayed an interspecific variability similar to *matK* and higher than that of *rbcL*. The addition of *ycf1* to the core barcode that includes *matK* and *rbcL* shows more robust and effective results in *Cycas*. The main drawback of the DNA Barcoding analysis using *ycf1* is the lack of sequences in public databases; for this reason, its efficiency as DNA barcode could be underestimated.

1) CBOL Plant Working Group (2009) Proceeding of the National Academy of Sciences, 106, 12794-12797.
2) F. Nicolalde-Morejón, F. Vergara-Silva, J. González-Astorga, D.W. Stevenson, A.P. Vovides, V. Sosa (2011) Cladistics, 27, 150-164.
3) W. Dong, C. Xu, C. Li, J. Sun, Y. Zuo, S. Shi, T. Cheng, J. Guo, S. Zhou (2015) Scientific Reports, 5, 8348.

## 4.1 = Microplastic pollution affect sediment microbial community structure: evidence from the Volturno River (Southern Italy)

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The sources, abundance, and ecological implications of microplastic (MP) pollution in rivers are unknown and probably substantial. We explored them in the Volturno River (Southern Italy) by investigating the concentration of Mps in sediments collected along the watercourse. Samples were analysed by the Polymer Identification and Specific Analysis (PISA) protocol, to quantify the total mass of individual polymer types present as microplastic particles: the sediments were sequentially extracted with selective organic solvents and the polymer classes polystyrene (PS), polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polycarbonates (PC), Nylon 6 (PA6) and Nylon 6,6 (PA66) were quantified by pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) and HPLC. Furthermore, in the light of current evaluations of the impact of anthropogenic activities in natural habitats through the study of their microbial diversity, we investigated the 16S and ITS metagenomics of Volturno river using next generation sequencing in Ion Torrent™, to explore bacterial and fungal taxonomy and ecological dynamics of sediment samples. We detected MPs in all samples taken from the study area. The total concentration of MPs ranged from 1.05 to 14.55 ppm and identified two distinct population data: high-MP contaminated and low-MP contaminated sediments. Overall, PP and PET were the dominant polymer types. According to the Polymer Hazard Index (PHI), the risk of MP pollution of the analysed sediments was categorized as Hazard level III/IV (corresponding to Danger category). Metagenomic data revealed that the presence of MPs significantly affects the abundance of microbial taxa, evidencing *Flavobacteraceae* and *Nocardiaceae*, known to degrade polymeric substances, in high-MP contaminated sediments. ITS metagenomic results highlighted that, at phylum level, Ascomycota occurred in all sediment samples. At the family level, it was worthy of note the presence of *Pleurotaceae,* since many species belonging to this taxa are known to degrade plastic polymers. This study provides new insights of ecological relevance related to MP pollution and suggests priorities for the management of one of the main rivers of Southern Italy. In addition, the study highlights that monitoring programs of river ecosystems should address also bacterial communities.

## 4.2 = Impacts and management of invasive *Carpobrotus* in Mediterranean island ecosystems: experiences from projects RESTO CON LIFE and LIFE LETSGO GIGLIO

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Biological invasions represent one of the most dramatic threats to biodiversity, contributing substantially to the widespread and accelerated decline in Earth's biodiversity and associated benefits to people. The species from the *Carpobrotus* genus are well-known invasive plants native to South Africa, whose detrimental effects on native communities are widely documented. These impacts have become particularly important on small Mediterranean islands, where they often threaten coastal habitats and endemic species. Due to these features, these species have been targeted by several projects of control (1). Within this contribution, we report the experiences of control of *Carpobrotus* conducted in the Tuscan Archipelago within the EU LIFE projects RESTO CON LIFE "Island conservation in Tuscany, restoring habitat not only for birds", on the island of Giannutri and LIFE LETSGO GIGLIO "Less alien species in the Tuscan Archipelago: new actions to protect Giglio island habitats" on the island of Giglio, focusing on the dynamics of vegetation recovery after the interventions.
In both projects, it was adopted an integrated approach with manual removal and covering with mulching sheets. At Giannutri, the intervention areas included a mosaic of rocky cliff coastal vegetation including the habitats of the Vegetated sea cliffs of the Mediterranean coasts and *Salicornia* and other annuals colonizing mud and sand, and disturbed areas with loose soil close to the cliffs. At Giglio the habitats most affected by the presence of this species include the Vegetated sea cliffs of the Mediterranean coasts with endemic *Limonium* spp., the Halo-nitrophilous scrubs (*Pegano-Salsoletea*) and the Low formations of *Euphorbia* close to cliffs. In both islands we surveyed the changes occurring to vegetation due to the control actions, adopting a Before-After-Control-Intervention design on permanent square plots of 2 meters of side, in invaded and uninvaded areas, in each of the habitats mentioned.
At Giannutri, the results obtained from five years of monitoring using permanent plots showed that *Carpobrotus* spp. exerted strong impacts on the invaded plant communities, with significant depletion and replacement of native species. The control methods proved to be effective in the suppression of the mat formed by the invasive species and led to a positive response of native vegetation. Nevertheless, the recovery of native plant communities differed depending on the type of substratum, taking longer on rocky cliffs due to the harsher conditions. Moreover, the recovery of native vegetation seems to be driven by nitrophilous species, especially in the plots treated with mulching sheets (2). At Giglio, the preliminary results clearly show impacts on plant alpha diversity (intended as the species diversity within each sampled plot) in all the habitats investigated in terms of a decrease in species richness, Shannon index, and abundance. Invaded communities also showed a severe change in species composition with a strong homogenization of the floras of the three habitats. Finally, the negative effect of invasion emerged even through the analyses of beta diversity, with *Carpobrotus* replacing a large set of native species (3). Preliminary results of the first vegetative season after the main intervention show a recovery of native species richness mainly for the Low formations of *Euphorbia* close to cliffs.

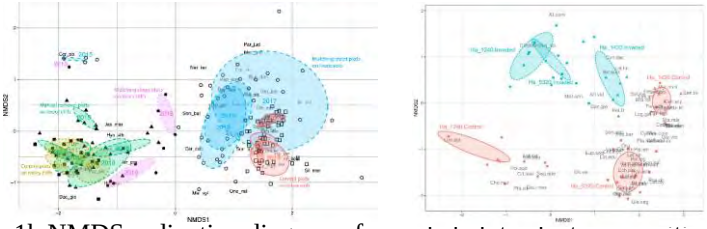

Fig. 1a-1b NMDS ordination diagram of sampled plots plant composition at Giannutri and Giglio.

1) J.G. Campoy, A.T.R. Acosta, L. Affre, R. Barreiro, G. Brundu, E. Buisson, L. González, M. Lema, A. Novoa, R. Retuerto, S.R. Roiloa, J. Fagúndez (2018) Bot. Lett., 165(3-4), 440-475.
2) L. Lazzaro, M. Mugnai, G. Ferretti, F. Giannini, M. Giunti, R. Benesperi (2023) Biol. Invasions, 2023 (online), 1-15.
3) M. Mugnai, R. Benesperi, D Viciani, G. Ferretti, M. Giunti, F. Giannini, L. Lazzaro (2022) Plants, 11(20), 2802.

## 4.2 = Role of root exudates in allelopathy-led interactions between alien and native woody species

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Allelopathy is a common invasion mechanism across the plant phylogeny, present in every lineage – monocots, dicots, gymnosperms (1). It has been assigned as a crucial factor in forest ecosystems structure and functioning as trees constitute the majority of plant biomass and release allelochemicals for long periods. The latter may accumulate in soil to toxic levels causing the failure of natural regeneration and the reduction of dominant species in the forests. Furthermore, highly productive alien invasive tree species may increase accumulation of allelochemicals in soil due to the inability of the local microflora to degrade them (1). Allelochemicals may be released into the environment from trees through stem flow, root exudation, leachates from aerial parts, volatiles from leaves, and litter decomposition. In the majority of cases, allelopathy of donor plants is assessed by testing the effects of extracts and leachates on germination and early seedling growth on selected receiver plants. The latter are usually model plants with rapid and homogeneous germination rates, convenient for the experimental design but providing results challenging to scale to natural conditions. Most examples of allelopathy in trees are associated with alien species that are rapidly becoming dominant in their new habitats (2). Invasive woody plants have been demonstrated to have flavonoid richness in roots higher than non-invasive ones, especially flavonols and flavones. The higher flavonoid richness in invasive woody species may explain why they tend to be less selective with their fungal symbionts than natives (3).
We attempt to summarize the main findings regarding allelopathy, particularly in woody-woody interactions and related allelochemicals identified, focusing on the role of root exudates in allelopathy. Subsequently, we revise the methodological approaches commonly used in experimental designs. Finally, we present several proposals for the terminology in allelopathy and identification-quantification of putative allelochemicals in woody species.

1) S. Kalisz, S. L. Kivlin, L.B. Murphy (2021) Biol Invasions, 23, 367-371.
2) V.G. Lebedev, K.V. Krutovsky, K.A. Shestibratov (2019) Molecules, 24, 1636.
3) V. Borda, K.O. Reinhart, M.G. Ortega, M. Burni, C. Urcelay (2022) Biol Invasions, 24, 2757-2768.

## 4.2 = Comparing the impacts of the two alien species (*Carpobrotus*, *Opuntia stricta*) on plant and invertebrate communities in small Mediterranean islands

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*Carpobrotus* spp. and *Opuntia stricta* are important invasive alien plants (IAPs) in Mediterranean habitats, particularly on in island systems. Despite the negative ecological impacts of *Carpobrotus* spp. on soil and vegetation have been widely documented, their impacts on invertebrate communities are poorly understood. From the few studies undertaken, an increase in the species richness of beetles and spiders has been observed after the removal of this invasive species (1, 2). As regards *Opuntia stricta*, there are as well few studies on its impact on invertebrate communities, in favour of others more focused on its socioeconomic impacts and on plant diversity loss. Contrary to what has been found for *Carpobrotus* spp., *Opuntia stricta* seems to not significantly affect spider communities in terms of species richness, density or assemblages, but it does significantly affect beetle assemblages (3). Our study aims to assess the impacts of these IAPs on native plant and invertebrate communities in Giglio and Capraia, two small islands of the Tuscan Archipelago (Italy).

Thanks to data previously collected in the Tuscan Archipelago, it was possible to plan a consistent sampling design for both islands. For what concerns Giglio Island and *Carpobrotus* spp., we created an experimental design involving the two most invaded areas of the island, Fenaio, to the north, and Capel Rosso, to the south. We randomly launched 18 square plots of 4 $m^2$ size, 6 in the invaded area, 6 in the uninvaded area and 6 where the species were manually removed one year before. All of these plots were located within habitats of conservation interest and at least 25 meters apart from one another. For each of them, we recorded data on plant species occurrence, their abundance. Furthermore, we collected soil samples for the Berlese funnel method and for soil microbiota analysis and sampled ants using pitfall traps.

As regards Capraia Island and *Opuntia stricta* species, we randomly launched 12 square plots of 4 $m^2$ size, 6 in invaded areas and 6 in uninvaded ones. Then, we recorded plant species occurrence, their abundance and collected the soil samples for Berlese analyses and ants as described above.

Preliminary results show a decrease in plant species richness in the invaded areas compared to the uninvaded ones in both cases. We detected a greater loss of plant species in the case of *Carpobrotus* spp. invasion than in the case of *Opuntia stricta* invasion, given the ability of the former to build suffocating monospecific mats. As regards the impacts on the pedofauna, *Opuntia stricta* does not seem to change completely soil structure and invertebrate communities, while *Carpobrotus* spp., consistently with the literature, significantly modify edaphic properties and invertebrate communities. Future prospects include assessing impacts on other invertebrate groups such as mollusks.

1) J. Braschi, O. Hélard, C. Mazzia, P. Oger, P. Ponel, E. Buisson (2021Biodiversity and Conservation 30, 497-518.
2) J. Braschi, A. Torres, S. Fadda, E. Buisson, P. Ponel (2021) Restoration Ecology, 29(5), e13387.
3) M.P. Robertson, K.R. Harris, J.A. Coetzee, L.C. Foxcroft, A.S. Dippenaar-Schoeman, B.J. van Rensburg (2011) South African Zoology, 46(2), 205–223.

## 4.2 = Damages on local vegetation caused by the mouflon population on two islands of the Tuscan Archipelago, Giglio and Capraia islands

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On a global scale, it is known that ungulates can conspicuously modify the environment they live in. Moreover, the European mouflon, an artiodactyl mammal belonging to the Ungulates, is considered a species with high potential impacts on the local vegetation and fauna of its home areal. The main mechanisms of impact of mouflon could be: direct effects related to the grazing of individuals of plant species, which can lead to a rarefaction of already rare or conservation-worthy species; and indirect effects, related to the transformation of environments, for example through the opening of lanes and the disruption of the topsoil. Commonly, significant impacts are visible on grassland and shrub areas, caused by trampling and the modification of the dynamics of renewal of natural populations due to the removal of young seedlings and shoots, which could also have long-term repercussions on both local flora and fauna. The aforementioned impacts are also observed on the islands of the Tuscan archipelago that host alien mouflon populations. This study focuses on the impacts caused by mouflon on the Giglio and Capraia Islands, which are at different stages of mouflon impact control. In fact, on both islands, vegetation monitoring has been carried out since 2020, however, only on Giglio Island, efforts to eradicate mouflon from the island have begun, as part of actions C.1 "Eradication of the mouflon" of the project LETSGO GIGLIO.
LETSGO GIGLIO “Less alien species in the Tuscan Archipelago: new actions to protect Giglio island habitats” is a Life Natura project co-funded by the European Commission (www.lifegogiglio.eu). The project ensures the collaboration of the Tuscan Archipelago National Park, the Department of Biology of the University of Florence, and the Company NEMO srl, for different actions on Giglio island (Tuscan Archipelago) towards the conservation of habitats protected under Dir. 92/43/EEC, thanks to the reduction of important threat factors, mainly represented by invasive alien species.
Since 2020 and on both islands, as part of the monitoring of damages on local vegetation, the impacts linked to mouflon were monitored through the survey of twenty randomly extracted transects, carried out in holm oak woodland, tall scrub and low scrub/garrigue habitats. In each transect, all individuals of woody species were counted, recording any evidence of grazing according to a simple four-level impact assessment scale (absent, low, medium, and high), and a subdivision between adult individuals, shoots, and seedlings.
This contribution will show the results of the monitoring carried out to date, highlighting that the greatest impacts observed in habitats protected by the "Habitats Directive" (Dir. 92/43/EEC) and especially for the Mediterranean shrublands and holm oak forests (hab. 9340 *Quercus ilex* and *Quercus rotundifolia.* On Giglio island, the first two years of monitoring were carried out before the intervention on the mouflon population, giving an important view of the initial condition of the vegetation affected by the grazing. On both islands, the results show a greater load of grazing in correspondence to where the most of population is located, with a distinct predilection for the consumption of *Quercus ilex, Arbutus unedo* and *Erica sp.* shoots. These results highlight a potential impact on the renovation of the most evolved woody communities. Furthermore, we found less impact on the vegetation where the presence of low scrub and garrigue is more significant. During the most recent monitoring campaign carried out in May 2023, we found a visible diminishing of damages to vegetation in Giglio Island, which could confirm the effectiveness of the actions carried out to date.

## 4.2 = Impact assessment of four invasive alien plant species in Croatia

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The importance of being able to assess, compare, and forecast the extent of impacts caused by various alien species has been acknowledged for a considerable time. This capacity is crucial for identifying and prioritizing suitable measures to address the situation (Hawkins et al., 2015). Risk Assessment is a systematic approach to assess the scale and likelihood of arrival, establishment, spread, and impact of alien species to identify those that are likely to become invasive. The approach is further developed through the standardised assessment of species impacts including positive and negative aspects of species' presence.
Different assessment protocols have been developed to evaluate the current and potential impacts of non-native species, but consistency among them has received limited attention. We studied the EU and other publicly available protocols, adapted for the invasive alien plant species (IAPS) and area of Croatia, and created our customized protocol. It includes questions about the species, the likelihood of its introduction, establishment, and spread in a new habitat, the potential for the species to provide ecosystem services, adverse effects on the environment, plant, and animal species, and adverse socioeconomic effects, as well as management of the species. Our protocol was developed considering the beneficial aspects of IAPS and will be tested by selected local and international experts. We applied the protocol to the assessment of the following species: *Helianthus tuberosus* L., *Ailanthus altissima* (Mill.) Swingle, *Robinia pseudoacacia* L. and *Solidago canadensis* L.
The results of the assessment pointed out the numerous potentials of IAPS such as a source of specialized metabolites with possible use in phytopharmacy. Assessment accompanied with the phytochemical profiling can be useful tools in successful IAPS management.

1) C.L. Hawkins, S. Bacher, F. Essl, P.E. Hulme, J.M. Jeschke, I. Kuhn, T.M. Blackburn (2015) Diversity and Distributions, 21, 1360-1363.

## 4.2 = Alien plant invasion during early succession stages of dune systems is driven by soil properties

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Biological invasion is nowadays recognised as one of the major threats to biodiversity (1). This is particularly true for coastal habitats, where dunes are considered one of the most invaded habitat worldwide. Many studies linked the success of alien plant invasion in dune ecosystems to human disturbances, but less is known about the role of soil properties in plant invasion. Soil properties can directly affect plant dune colonization and the final community composition (2,3). This is expected to be linked to the interplay of soil and the plant functional response, generating important plant-soil feedbacks able to reverberate on the community structure and composition. Plant-soil interactions are thus expected to be crucial in explaining the invasion processes, but their role in dune alien colonization in mostly unknown.
We performed a manipulative experiment in a barrier island of the Marano and Grado's lagoon, Northern Adriatic Sea. The whole plant community of backdune was erased by a soil miller in the selected plots to trigger a new ecological succession and test the mechanism of alien plant invasion during the early stages of plant colonization. In 8 experimental bocks, we altered soil properties by adding salt, nitrogen and organic matter (i.e. peat) and combining those treatments in 1 m2 plots with a factorial design (i.e. 8 replicates × 8 treatments = 64 plots). We recorded the emergence of seedlings with a camera system every 15 days. At the end of experiments, we recorded the plant community composition and measured the following traits: plant height, species cover, number of individuals, leaf pigments, SLA. Moreover, those traits were calculated for the overall community and for the key species (*Cakile maritima* Scop.) We also estimated the decomposition rate of the soil using the Tea Bag Index. In addition, the same parameters were collected in 8 reference plots (i.e. surrounding unaltered plant community).
The results showed that most of the treatments decreased the species richness of alien plants, in particular where soil salt content was enhanced. Moreover, some treatments had positive effects on the native plant cover and decreased the overall number of alien individuals, potentially reducing the initial propagule pressure due to the soil seed bank and, hence, changing the further plant community trajectories. This study provides new information on conservation and management efforts in this ecologically sensitive area, giving new insight into the dynamics of biological invasion and the impacts on native ecosystems.

1) F. Lami, S. Vitti, L. Marini, E. Pellegrini, V. Casolo, G. Trotta, M. Sigura, F. Boscutti (2021) Ecological Indicators, 133, 108450.
2) M. Vilà, C. Basnou, P. Pyšek, M. Josefsson, P. Genovesi, S. Gollasch, W. Nentwig, S. Olenin, A. Roques, D. Roy (2010) Frontiers in Ecology and the Environment, 8, 135–144.
3) S. Vitti, E. Pellegrini, V. Casolo, G. Trotta, F. Boscutti (2020) Journal of Plant Ecology, 13, 667–675.

## 4.2 = The walking of *Salpichroa origanifolia* in Pisa city and surroundings and its dispersal by birds

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*Salpichroa origanifolia* (Lam.) Baill. (Solanaceae) is native to South America and it is well known as an invasive species. It is present in Africa, North America, Australia and Europe.
This species is a fruticose chamaephyte, scrambling sub-shrub. It has been abundantly described (1) as an expanding species in the Canton of Ticino. In Italy it is widespread in almost all regions. For Tuscany it is reported as naturalized, but we believe it could be defined as invasive.
Several observations are reported for Tuscany. Its presence has been known in the province of Pisa since 1981 (2). According to Wikiplantbase#Tuscany (http://bot.biologia.unipi.it/wpb/toscana/index) has been widespread in the region since 1999 and it is found in the provinces of Florence (municipalities of Castelfiorentino, Fiesole, Florence), Grosseto (Castiglione della Pescaia), Livorno (Livorno, Rosignano Marittimo), Lucca (Pietrasanta), Massa Carrara (Massa, Montignoso), Pisa (Pisa, San Giuliano Terme, Tirrenia). It colonizes very different environments (synanthropic areas, vegetable gardens, canals, roadsides, thermophilic woods, holm oak woods with stone pine *Pinus pinea* L., etc.).
The introduction of *S. origanifolia* is usually due to cultivation, but it is also caused by animals (1).
In and around Pisa it is found along roads and in anthropic environments (Fig. 1a, b). Its distribution and development are strictly related to the ruderal environment, where the plant can suffocate even tall trees (see Tirrenia) (Fig. 2); birds are thought to be responsible for its spread (Fig. 3).
Birds are known to play a key role in the dispersal of the species. The involvement of some species of passerine birds, such as *Zonotrichia capensis* and *Poospiza whitii*, has been highlighted in studies conducted in the South American countries of origin of *S. origanifolia*. Seeds of the plant were found in the faeces of these birds, and their germinability was confirmed (3).
Occasional observations relating to the city of Pisa proved this attitude in the Italian Sparrow *Passer italiae* and the Wood Pigeon *Columba palumbus*. Further studies are underway to determine which bird species are attracted to *S. origanifolia* fruits, in addition to those mentioned.

Fig. 1a-1b. *Salpichroa origanifolia* in Pisa and Tirrenia.

Fig. 2. *Salpichroa origanifolia* in Tirrenia.

Fig. 3a. Flower and fruit of *Salpichroa origanifolia.*

Fig. 3b. Italian Sparrow, male.

1) S. Mangili, N. Schoenenberger, P. Selldorf, I.Sasu, C. Haritz, A. Borsari, B. Marazzi, D. Frey (2020) Bollettino della Società Ticinese di Scienze Naturali, 108, 83-91.
2) L. Viegi, G. Cela Renzoni (1981) C.N.R. Progr. Final. «Promozione della qualità dell'ambiente»: AQ/1/132, pp. 97.
3) C.A. Reati (2022) Ornitología Neotropical, 182-191.

## 4.2 = Importance of communication of invasive alien species: the case of dissemination events in the project LIFE LETSGO GIGLIO

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The Project LIFE LETSGO GIGLIO "Less alien species in the Tuscan Archipelago: new actions to protect Giglio island habitats" aims to improve the quality and natural character of the ecosystem present on Giglio Island by protecting the habitats and some of the species that live in them, in line with the provisions of Directive 92/43/EEC (Habitats Directive). One of the greatest threats to these habitats is the expansion of invasive alien species, including *Carpobrotus* spp. (*C. edulis* and *C. acinaciformis* both present on Giglio Island). These species are native to South Africa, widely distributed and known for their invasive behavior in the Mediterranean basin. The presence of *Carpobrotus* spp. determines negative impacts on invaded ecosystems due to its rapid growth and mat formation, preventing the growth of native species due to soil cover, the modification of its composition and the allelopathic effect on litter, leading to a reduction in biodiversity (1). Along with the actions carried out for the containment of *Carpobrotus* in Giglio Island, including interventions of manual removal, and covering with mulching sheets, among the main objectives of the LIFE LETSGO GIGLIO project is also to communicate the need of improving the conservation status of habitats and the species that inhabit them, through dissemination activities. With the aim of transferring the replicability of the removal experience (techniques and operating methods) to individuals who will represent experts and technicians in the area in the future, involving university students, a number of demonstration events for the removal of *Carpobrotus* spp. are included in the communication plan.
The events were held in May 2022 and 2023 and there was involvement of around 40 students from the Universities of Florence and Siena each year. These events included a presentation on the problem of invasive alien species and the project's aim and interventions. Moreover, they foresaw the visiting some areas targeted by the project interventions, and on the second day, the students themselves were involved in the removal of Carpobrotus, in selected areas. At the end of the activities, a questionnaire was proposed to check the understanding of the problems caused by invasive alien species, to get feedback about the experience and the participants' perception of the problem and threats to biodiversity. The feedback was positive, both in the understanding of the proposed topic and in the practical usefulness of the activity conducted. The answers show that all students can define what invasive alien species are and they know what problems are related to their presence. They recognize the usefulness of prevention activities and direct interventions on plant species while there are different opinions regarding animal removal interventions.
These events represent pivotal tools to enhance the effectiveness and validity of the dissemination and communication efforts of the project and are fundamental both in attracting an interest in the specific problem of habitat protection, in this case in the Islands of the Tuscan Archipelago, but also and especially in raising awareness about the problem of invasive alien species in general.

Fig. 1. Manual removal of Carpobrotus by the students during the event in 2023.

Fig. 2. Students visiting areas targeted by the project interventions in 2022.

1) M. Mugnai, R. Benesperi, D Viciani, G. Ferretti, M. Giunti, F. Giannini, L. Lazzaro (2022) Plants, 11(20), 2802.

## 4.5 = An ecological study of *Tuber magnatum* for the regeneration of rural areas

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Truffles are edible fungi, belonging to the *Tuberaceae* family. Among them, there are many species with great economic value. Indeed, several rural realities have made truffles a source of livelihood. One of the most appreciated for its flavors is the white truffle (*Tuber magnatum* Picco). Due to the inability to successfully cultivate this species like other truffles, its production remains more akin to that of wild resources. The decline in white truffle yields can be attributed to climate change, the abandonment of productive areas, and increased harvesting pressure. Consequently, studies of that sites are crucial to understanding how to intervene to preserve them. More attention is also needed because these areas are limited to Italy, France, and some Balkan regions. This study was carried out in two areas, Italy and Croatia. The aim is to describe different environments where the *Tuber magnatum* grows and which characteristics are indispensable to understanding whether a site is likely to be productive or not. All this will lead us to find out how and where we can intervene to safeguard production. The area in Italy is located in Tuscany, in San Giovanni d'Asso (Fig. 1). It is a valley floor where the dominant host species are *Populus canescens* (Aiton) Sm., *Quercus cerris* L., and *Salix alba* L. The area examined in Croatia is the Motovun Forest (Fig. 2). This is a location where in ancient times the Venetians exploited oaks (*Quercus robur* L.) for the manufacture of galleons. It is now a Natura 2000 site and protected area as a Special reserve of forest vegetation (1). Over time, diversions of the river course were implemented to avoid flood damage. This is also affecting truffle production. Our study consists of a chemical-physical and genomic soil analysis and a vegetation analysis. With the data obtained, we will be able to learn more about the fundamental ecological characteristics of the white truffle.

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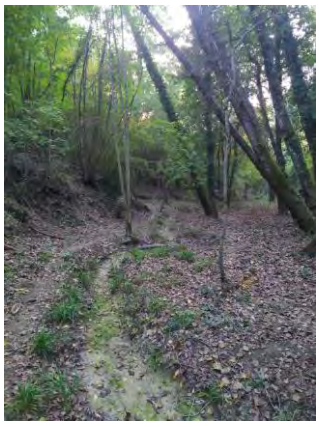

Fig.1. Valley floor in San Giovanni d’Asso, Tuscany, Italy.

Fig. 2. Motovun Forest, Croatia.

1) J. Vukelić, P. Korijan, I. Šapić, A. Alegro, V. Šegota, I. Poljak (2018) South-east Eur. For., 9(1), 1-16.

## 4.7 = Population changes in an autochthonous microalgae consortium during preliminary phytoremediation tests of urban wastewaters for future on-site applications

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Environmental pollution from wastewaters (WW) produced by human activities is a well-known problem that cause, among others, biotic pollution and an excessive release of nitrogen (N) and phosphorus (P) into natural waterbodies, which can lead to eutrophication. As reported by [1], nearly 30% of the overall population in Central and Eastern European countries lives in small towns (< 2.000 inhabitants) that are not connected to a WW treatment plant (WWTP). WW produced by these settlements can significantly contribute to pollution if untreated. As a solution, low-cost on-site WW treatments are preferred. Italian legislation only regulates the quality of effluent from WWTP for more than 2.000 Population Equivalent (P.E.) and lets the local regional authorities choose the limits for smaller WWTPs [2]. These limits usually concern only organic matter ($BOD_5$ and COD), total suspended solids and N in the form of ammonium. However, other nutrients, such as P as phosphate and N as nitrate, can cause pollution. As an on-site solution, microalgae can be proposed to remove nutrients from WWs producing biomass, that can be used for many biotechnological applications.
In the present study, partially treated WWs, supplied by Hera-Ferrara and collected from the oxidation tank of a small WWTP located at Montesanto (FE; 400 P.E.) in November 2022 and February 2023, were used for preliminary phytoremediation tests. The initial chemical analyses showed that both WW had low nitrate and phosphate concentrations, but ammonium was above the limitation imposed by law (25 mg $L^{-1}$ [2]). Therefore, both effluents needed further depuration. To avoid biotic pollution, a microalgae consortium, composed of Scenedesmaceae-like (≈65% of the total population), *Chlorella*-like (≈34%) and *Nitzschia*-like (≈1%) algae, was collected from the November effluent and used for experiments. The consortium was inoculated in the February effluent under laboratory conditions and microalgae growth and productivity, morpho-physiological aspects and nutrients removal were monitored for 21 days. Results showed that cell density tripled in the first 8 days of cultivation and reached value 6-times the initial density by the end of the experiment. Interestingly, the composition of the population changed over time, resulting in a higher percentage of *Chlorella*-like cells (around 60% of the total population) by day 2 and a higher percentage of Scenedesmaceae-like cells (around 70%) at the end of the experiment. *Nitzschia*-like algae were no longer observed. Regarding nutrients, almost total phosphate depletion was achieved by day 8 and, in the same time, the ammonium concentration was far below the limit imposed by Emilia Romagna legislation. Concomitantly, the already low nitrate concentration did not change significantly.
The rapid nutrient removal, along with the high biomass production, are promising results that open the possibility for further research and future on-site applications of the isolated microalgae consortium.
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1) Sustainable Sanitation in Central and Eastern Europe: Addressing the Needs of Small and Medium-Size Setlements, I. Bodík (2007) GWP Central and Eastern Europe: Bratislava, ISBN 978-80-969745-0-4.
2) Direttiva Concernente Indirizzi per l'applicazione Del DLgs 11 Maggio 1999 n 152, Come Modificato Dal DLgs 18 Agosto 2000 n 258 in Materia Di Tutela Delle Acque Dall'inquinamento. (Pubblicazione - Bollettino Ufficiale Regione Emilia Romagna - Parte Seconda n. 88 Del 24 Giugno 2003).

## 4.7 = Characterization of leaf functional traits and atmospheric particulate matter retention performance by Mediterranean plants potentially used for the design of roadside vegetation greening

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Six plants typical of the Mediterranean basin area (*Myrtus communis, Nerium oleander, Olea europea, Pinus halepensis, Rhamnus alaternus, Rosmarinus officinalis*) were studied in their functional leaf traits to assess the retention capacity of PM10 and PM2.5 atmospheric particulate matter generated by vehicular traffic along a transect of a highway in Southern Italy. Potted plants, of which LAI *Leaf Area Index* was calculated, were exposed at two locations along the road section for two successive time intervals, a first period of 15 days and a second period of 16 days, in August 2021. Air monitoring through a mobile air sampler was conducted at the same exposure points to have a direct correlation of phytoremediation with air pollution. The six species exhibited were studied by scanning electron microscopy (SEM) for evaluation of functional traits of the sclerophyllous leaves, such as the presence, density e morphology of trichomes, presence of stomata and their characteristics, presence of cuticle and its thickness that act as a trap for particulate pollutants. The PM absorptions of the leaves were estimated through filtrations of water and chloroform washing solution to obtain the mass of particulate adhered to the surface of the leaf and trapped in the cuticular wax layer. The PM uptake results obtained about the saturation capability of leaves will allow to estimate which plants, and which combinations of plants, characterized by specific characteristics of their canopy will be more promising in the retention of these dangerous contaminants, so that they can be used for the design of the greenery surrounding the high-traffic roads.

## 4.7 = Potential and innovation of a constructed wetland pilot plant in an internal area of Alexandria of Egypt: use of a green biotechnology for the treatment and safe reuse of civil wastewater

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In Egypt, approximately 833 million hectares of agricultural land worldwide are saline, mostly in arid or semi-arid regions; more than 1.5 billion people are thus threatened by soil degradation and loss or limitation of food production (FAO, 2022). The Mediterranean basin is experiencing the effects of global warming more rapidly than the rest of the world (UNEP, 2020); the management of freshwater resources is therefore a critical issue in the development and adaptation to climate change of the Egyptian population concentrated especially next to the Nile delta. The SRTA-City working group implemented a Nature-Based Solution (NBS), consisting of a septic tank completed by a sand biofilter, in a village at Banger EL (Alexandria), for the treatment of civil wastewater, in order to reuse the remediated water for soil washing. Unfortunately, the effluent doesn't yet comply with legal standards required for its reuse in agriculture. In this context, the use of phytoremediation (PR) technology as finishing treatment, through the implementation of Constructed Wetlands (CWs), represents one of the most exploitable alternatives for local communities, thanks to their environmental and economic sustainability. To this purpose, based on the previous experiences of the UNISA team (1), we realized an innovative PR pilot plant, of submerged horizontal flow and semi-hydroponics. Therefore, we tested the efficiency of the CW on the effluent coming from the NBS system. The pilot plant has been carefully dimensioned and the plant species (*Phragmites australis*, *Thypa* spp., *Arundo donax* and *Nerium oleander*) were chosen among the ones available on site. In order to overcome PR applicability limit due to the large surfaces needed, three strategies have been adopted to make the PR plant more efficient in biological terms: the use of natural filling media with high Specific surface (Ss); acclimatation by recirculating in the filter bed an aqueous solution enriched with rhizosphere microorganisms from the selected plants; installation of a bioelectric cell to enhance the biodegradation of organic matter without supplying energy. The efficiency of the PR plant was evaluated by analyzing several parameters, as Bio/Chemical Oxygen Demand ($BOD_5$ and COD), Nitrogen compounds, etc. After 48 hours treatment, a significant reduction of all pollutants was observed (*e.g.*, 79% remediation efficiency for COD), which were almost totally removed after two weeks, complying with the Egyptian limits for the effluent reuse in crop irrigation. In addition, about one order of magnitude removal of pathogenic colony-forming unit for mL (*E. coli*, Staph, Yeast) was observed in the CW treated water, which can be completed by a solar-driven UV lamp installed downstream. Moreover, toxicity and germinability tests with the model organism *Daphnia magna* and tomato seeds, respectively, showed an increased mortality caused only by the CW influent and not by the effluent. Therefore, the technical innovations adopted have certainly contributed to the achievements of objectives, reducing the marginalization of Mediterranean inland areas.

1) I. Spiniello, M. De Carluccio, S. Castiglione, E. Amineva, N. Kostryukova, A. Cicatelli, L. Rizzo, F. Guarino (2023) J Environ Manage, 331, 117211.

## 4.8 = Extensive Green Roofs (EGR) and the Five Ws: origin and evolution, aims, approaches, and botanical view

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The growth of urbanization affects both developed and developing countries, representing one of the most critical social and economic phenomena that can promote the overall development of different aspects of human life. Cities of the future should strive to be greener through the design and management of green infrastructure that can aid urban resilience and livability [1]. Even if urban forests and wider green spaces in the cities are essential, a limit of ground-level spaces for nature-based solutions often exists. Then, the construction of green roofs could represent an excellent supplementary space. Extensive Green Roofs (EGRs) are one of the nature-based solutions that can provide benefits such as climate change mitigation, improved human health and well-being as well as social and economic benefits [2]. Making use of a journalistic approach, based on the 5 W's, this review of about 1280 scientific papers was aimed to offer answers regarding the origin, development of interest, and how these important green infrastructures have been studied. Special attention was also paid to how the plant selection was addressed. The review showed that the first modern interest in the EGRs study dated back in the first decades of 1900s with the new trends of contemporary architecture in Europe. Their employment later started from Germany and Swiss, followed by America and Asia, with a continuous increment of interest in the last decades. The study was mainly approached with engineering and architectonic aims due to their functional benefits. At the same time, there is evidence of a limited collaboration among the involved disciplines and the lack of interdisciplinarity producing a lack of attention to the botanical aspect. Indeed, despite their ecological benefits, the literature showed that the selection and the study of plants, which is dominated by *Sedum* sp., still needs more attention.
Considering the wide worldwide information regarding EGRs, we can provide recommendations for considering such topic with a multidisciplinary focus. Engineering and architectural issues, aimed to economic and aesthetic reasons, are highly relevant, but ecological aspects should be improved in further studies. A botanical approach which can widen the selection of species, now dominated by *Sedum* species, can bring many benefits, and would make the green roof more functional and environmentally sustainable. Finally, following the politics of Switzerland and Germany, where incentives, regulations and guidelines were introduced to facilitate their implementation [3], this activity seems welcomed to facilitate the implementation and the maintenance of these important nature-based solutions over time.

1) S.A. Nitoslawski, N.J. Galle, C.K. Van Den Bosch, J.W. Steenberg (2019) Sustainable Cities and Society, 51, 101770.
2) G. Caneva, A. Kumbaric, V. Savo, R. Casalini (2015). Plant Biosystems, 149(2), 374-383.
3) C. Catalano, V.A. Laudicina, L. Badalucco, R. Guarino (2018) Ecological Engineering, 115, 15-26.

## 4.8 = Interactions between plant response to environment and fungal microbiome in developing maize silks in relation to mycotoxin risk

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Maize (*Zea mays* L.) is one of the most important crops worldwide both in terms of yield and land surface used, which are constantly increasing. One of the most critical stages for maize reproduction and seed establishment is the emergence of silk from cob: silks are particularly susceptible to environmental stresses and represent a preferential entry route for mycotoxin-producing fungi such as *Fusarium verticillioides* and *Aspergillus flavus* (1). Moreover, from elongation to senescence, silks become a sink organ enriched in nutrients (*e.g.* Non-Structural Carbohydrates (NSC)), and a crossway for various primary and secondary metabolites. In maize silks, these metabolites are expected to be significantly affected by environmental stress conditions (2) and by the maturation stage of silk tissues themselves, possibly affecting the fungal colonization of the ear tissues. In temperate regions of cultivated maize, also pathogen growth and mycotoxin production are thought to be affected by environmental factors, such as alterations in temperature, rainfall and humidity (3), which are strictly related to climate change.
The aim of this study is to investigate the impact of the environmental conditions on the fungal microbiome in maize developing silks at two different phenological stages. To do so, some eco-physiological parameters have been measured in 5 plots under contrasting climate conditions, and the complete fungal microbiome has been sequenced for each plot, both at the beginning of emergence and at the senescence of silks.
In this study we expect to get new insights into the interplay of the environmental conditions, *i.e.* precipitation and temperature, and phenological stage of silks in determining the fungal microbiome of maize silks. We do believe that climate-induced plant response might be pivotal in shaping the microbiome communities by favouring some fungal groups and disfavouring others during early silk colonization.

1) M.E.H. Thompson, M.N. Raizada (2018) Pathogens, 7(4), 81
2) T.L. Slewinski (2012) Journal Of Experimental Botany, 63(13), 4647-4670
3) N. Magan, A. Medina (2016) World Mycotoxin J., 9, 673–684.

## 4.8 = Chemical profile, physiological status and stress resistance in the liverwort *Lunularia cruciata* colonizing polluted mining tailings

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Past mining activities often left a legacy of improperly managed by-products in the form of heavy metals-polluted heaps and tailing dumps. The tailing dump of Barraxiutta (municipality of Domusnovas, South-West Sardinia, Italy, 39°22′05.82″ N, 8°36′28.46″ E − WGS84) is constituted by fine particles resulting from the extraction of Pb and Zn. After almost a century from the end of extraction activities, the tailing dump still present relevant content of Fe, Pb, Cd and Zn, and as a consequence it is scarcely colonized by plants (1). Heavy metals (HMs) are in fact strong abiotic stressors which negatively affect the structure and the integrity of biological membranes and organic molecules prejudicing crucial aspects of plants physiology such as photosynthesis. Some plant species are however able to colonize metalliferous environments occupying niches unsuitable for the majority of plants. This is the case of the liverwort *Lunularia cruciata* (L.) Dum. (Lunulariaceae family) which colonizes the Barraxiutta tailing dump forming dense carpets in the most shady and wet spots.

In order to study the adaptive mechanisms behind the presence of *L. cruciata* in such an extreme environment, we characterized the liverwort as regards *i*) pollutants' uptake (measured by acid digestion of plant samples followed by ICP-OES analysis of the obtained solutions); *ii*) photosynthetic efficiency (measured by Mini PPM 100 portable fluorometer on dark-adapted plants), and *iii*) chemical profile (obtained by GC-MS analysis) of the essential oil obtained by the steam distillation of fresh plant material. The same parameters were measured on control individuals of the same species growing in comparable ecological conditions but in absence of environmental pollution.

Experimental results showed the individuals colonizing the tailing dump (polluted individuals hereafter) containing considerable amounts of soil pollutants with respect to controls. To follow the content (expressed as mg/g on dry weight basis) of the most representative soil pollutants measured in control and polluted individuals respectively: Fe 45.92 ± 4.88 and 320.54 ± 65.66, Pb 0.54 ± 0.02 and 30.68 ± 5.64, Cd 0.00 and 0.2 ± 0.08, Zn 0.28 ± 0.04 and 50.04 ± 8.84 (mean values of three replicates ± standard deviation). Despite the uptake of phytotoxic elements, good levels of photosynthetic efficiency seem to indicate non-stressed polluted individuals. A possible explanation for *L. cruciata* resistance towards detrimental effects of environmental HMs could be found in the production of protective secondary metabolites by the liverwort. PCA carried out on essential oil chemical profiles (explaining in its first two dimensions 73.9% of data variability) confirmed the latter hypothesis since polluted individuals were plotted apart from controls, suggesting variations in essential oils composition in relation to the different growing conditions. More precisely, plants colonizing the polluted tailing dump presented increased relative content of sesquiterpenes in their essential oil (24.9% *vs* 11.98% of sesquiterpenes compounds in the essential oil of polluted and control individuals respectively). Sesquiterpenes are volatile isoprenoids which are described to have a role in plants stress resistance and adaptation to adverse environmental features (2,3). In the present study case too, sesquiterpenes could represent a key element in the adaptation of the liverwort *L. cruciata* to the polluted post-mining environment. Further studies, also directed to explore metabolomic profiles (both primary and secondary metabolites), will be needed to delve into the adaptive mechanisms behind the presence of this versatile liverwort in the extreme ecological context of the polluted tailing dump of Barraxiutta.

1) A. De Agostini, C. Caltagirone, A. Caredda, A. Cicatelli, A. Cogoni, D. Farci, F. Guarino, A. Garau, M. Labra, M. Lussu, D. Piano, C. Sanna, N. Tommasi, A. Vacca, P. Cortis (2020) Ecotoxicol. Environ. Saf., 189, 110018.
2) C.E. Vickers, J. Gershenzon, M.T. Lerdau, F. Loreto (2009) Nat. Chem. Biol., 5, 283-291.
3) F. Loreto, S. Pollastri, S. Fineschi, V. Velikova, (2014) Environ. Exp. Bot., 103, 99-106.

## 4.8 = Pollen forecast in Italy

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Numerous studies in the field of aerobiology have increasingly shown that airborne pollens depend on vegetation and environmental conditions, with considerable variations from one country to another, within regions, between cities of the same region, and even within the same city.
In Italy, pollen data are collected according to the European standard CEN/TS 16868:2019, a standardized methodology with the value of a protocol (guideline) or guideline: the samples are analyzed using an optical microscope, and are labor-intensive, time-consuming and require specialized personnel. The data, collected weekly in the form of pollen grain concentrations/$m^3$ of air, are statistically processed and used for the production of weekly bulletins, pollen calendars, or for the study of vegetation.
Among the applications of aerobiology, descriptive statistics are widely used to describe changes over the years in vegetation as a source of pollen emission, and the development of predictive and dispersion models.
Two reviews have recently been published (1,2) concerning precisely the modelling applied in the field of aerobiology, in which articles published from 1998 (availability of electronic journals) to 2016 were considered: out of a total of 253 models, 24 are related to the Italian territory and are validated only for the areas from which the data used for model development came and are not applicable in neighboring territories (3).
The statistical literature on forecasting/modelling techniques is growing very rapidly. New techniques applied to the most varied fields of research - economics, biology, medicine, engineering, sociology, purchasing behaviour - are appearing every day, making it possible to obtain increasingly precise forecasts. Why are forecasting models for pollen dispersion not applicable to the whole of Italy?
Generally speaking, the presence of pollen in the atmosphere is strongly influenced by the vegetation characteristic of the sampling site and is linked to a complex system of interactions that depends on geographical and meteorological factors mediated by biological factors, which together interact in the various phases of the reproductive process, pollen release, transport and deposition.
From a geographical point of view, Italy constitutes a very complex system, with an extremely diverse climate, topography, and vegetation throughout its territory: geographical position, different latitude, exposure, altitudinal gradient, different geological substrata and land use all contribute to the presence of a great variety of habitats, resulting in a biologically rich landscape. While this diversity contributes to the richness of our country's natural heritage, it also increases the complexity and difficulty of accuracy of forecasting models. To this must be added the action of meteorological factors that intervene at two successive moments: in the medium and long term in the phenological development of plants and thus influencing the ripening and production of pollen; in the short term in the mechanisms of pollen dispersal and transport.
Furthermore, pollen dispersal is also influenced by the physical characteristics of pollen particles, such as shape, density, size, in close dependence on the movement and turbulence affecting air masses.
The combination of these factors suggests the need for a greater number of pollen samplers on Italian territory and for an official reference standard, as is the case for the monitoring of pollutants, airborne particles with structural and aerodynamic characteristics that are totally different from pollen grains, but capable of giving rise to superimposable yet independent behaviour.

1) A.M. Vélez-Pereira, C. De Linares, J. Belmonte (2021) Science of The Total Environment, 795. https://doi.org/10.1016/j.scitotenv.2021.148783
2) A.M. Vélez-Pereira, C. De Linares, J. Belmonte (2022) Science of The Total Environment, 845. https://doi.org/10.1016/j.scitotenv.2022.157351
3) A. Travaglini, A. Miraglia, M.A. Brighetti (2019) Abstract Book The Mediterranean Palynological Societies Symposium 2019, 45.

## 4.8 = Sustainable cultivation of Greco grapevine passes through understanding plant water use strategies: an outlook from wood formation within the GREASE Project

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The increasing frequency and duration of drought events in the Mediterranean region is threatening grapevine growth and productivity. To design cultivation strategies and counteract the negative effects of climate change, it is necessary to evaluate the plasticity of plant functional traits in response to environmental stresses. Recently, dendro-ecological techniques have been successfully applied to grapevine to reconstruct the vine ecophysiological history, in relation to pedo-climatic variability and to cultivation practices, through the analysis of anatomical traits in vine's trunk wood rings, with a focus on the efficiency and safety of the water transport system against embolism phenomena (1). Grapevine has a ring-porous or semi-ring porous wood, characterized by a high heterogeneity in the vessel network allowing the modulation of water transport according to inter- and intra-seasonal variability of water availability in the soil. Recent advancements in Magnetic Resonance Velocity Flow imaging allowed reconsidering the role of narrow vessels not only as a back-up system when large vessels are embolized, but also for their significant contribution to water flow when transverse pressure gradients redirect flow from larger vessels to narrower (2). In vineyards, the microclimatic variability as well as the management techniques can have a deep influence on xylem traits which results in different vine water use efficiency. For a reliable evaluation of how wood functional traits are related to inter- and intra-annual environmental variability as well as to cultivation management, along tree-ring chronologies, it is necessary to know when exactly the wood ring is formed, through the analysis of xylogenesis.
We monitored xylogenesis in *Vitis vinifera* subsp. *vinifera* 'Greco' vines growing in an experimental vineyard in southern Italy within the GREASE project (Modelli sostenibili di coltivazione del vitigno Greco: efficienza d'uso delle risorse ed applicazione di indicatori della Footprint family), funded by the Campania Region through the Rural Development Programme 2014-2020, in the framework of improving grapevine productivity, resource use efficiency and resilience for the sustainable management of vineyards. Xylogenesis was monitored by sampling microcores bi-weekly from the main trunk of vines from three plots with different soil management: cover crops, natural coverage, and soil tillage. Thin sections of the cambial zone were analysed through microscopy to identify cambial cells and cells in different differentiation phases. Plant canopy was meanwhile monitored through UAV techniques in the three plots.
The overall analysis of data showed that the different soil management influences the timing of wood formation according to the shifting in vegetation indexes, suggesting a high plasticity in the control of water use in grapevine in response to water availability in the soil.

Fig. 1. Logo of the PSR-GREASE Project and microscopy views of grapevine wood section in the cell division and differentiation region.

1) V. De Micco, E. Zalloni, G. Battipaglia, A. Erbaggio, P. Scognamiglio, R. Caputo, C. Cirillo (2018) IAWA Journal, 39, 145-155.
2) M. Bouda, C.W. Windt, A.J. McElrone et al. (2019) Nature Communications 10, 5645. https://doi.org/10.1038/s41467-019-13673-6

## 4.8 = Impact of microplastics on the aquatic plant *Lemna minuta* and trophic transfer from producer to a primary consumer in a freshwater food chain

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Pollution by microplastics (0.1 µm-5 mm plastic fragments) is currently one of the major threats to the conservation of aquatic and terrestrial ecosystems worldwide (1). Although they are considered among the most alarming contaminants, toxic effects on plant organisms are unclear, particularly on freshwater plants (2).
This study aimed (i) to evaluate the toxic effects of polystyrene microplastics (MPs) on the aquatic plant *Lemna minuta*, and (ii) to test the possible trophic transfer of these microplastics from *L. minuta*, as producer, to aquatic larvae of the moth *Cataclysta lemnata*, as primary consumer that feeds on this plant.
*Lemna minuta* fronds were grown in different concentrations of microplastics of poly(styrene-co-methyl methacrylate) (MPs) (50, 100 mg/l) and exposure times (0, 7, 14 and 28 days). Phytotoxic effects of MPs were evaluated by analyzing morphological and biochemical parameters (frond and root size, growth, chlorophyll and malondialdehyde-MDA content). In addition, chemical and physical parameters (temperature, pH, dissolved oxygen, conductivity, salinity) of the aqueous medium in which the plant grew were analyzed. *Lemna minuta* fronds were observed under scanning electron microscopy (SEM) for assessing the possible adsorption of MPs on the fronds. SEM observations revealed the adsorption of microplastics on the fronds, with a preference for the abaxial surface. After 14 and 28 days of exposure, and at both concentrations, some parameters such plant growth and chlorophyll content showed a significant decrease compared with the control. In contrast, MDA values did not point out any alteration of oxidative lipid damage in plant tissues. The presence of MPs induced root elongation when compared to the control plants. The effects of MPs on *L. minuta* were more evident at T28. These results contributed both to better understand the impact of MPs on aquatic plants and to highlight that MP contamination causes chronic-type effects, detectable with longer exposure times than those traditionally used (7 days) in phytotoxic duckweed-based tests.
To evaluate the possible trophic transfer of MPs from producer to consumer in a freshwater food chain, *C. lemnata* larvae were fed with fluorescent MPs-contaminated *L. minuta* fronds. Using fluorescence microscopy, MPs were detected in both the gut of the larvae and in the feces, showing that *C. lemnata* larvae had ingested contaminated fronds. In addition, larvae fed with contaminated fronds showed high mortality rates (90%) and total inability to complete the life cycle.
The results showed that there was a trophic transfer of microplastics from producer to primary consumer along a freshwater food chain, generating negative effects on both aquatic plants and aquatic herbivores.

1) I. Azeem, M. Adeel, M.A. Ahmad, N. Shakoor, G.D. Jiangcuo, K. Azeem, M. Ishfaq, A. Shakoor, M. Ayaz, M. Xu, Y. Rui (2021) Nanomaterials, 11, 2935.
2) G. Kalčíková (2020) Environmental Pollution, 262, 114354.

## 4.8 = Classification of aerobiological stations based on their spatial representativeness

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An aerobiological monitoring network consists in a set of volumetric samplers for pollen and spores which serves to control and study, in all its multiple aspects, the biological component of the airborne particulate matter. This information is useful for pursuing objectives in the environmental field (integrating air quality monitoring, the estimation of the biodiversity of plant species, the detection of phenomena related to climate change) and in the health field (producing information in diagnostics, clinical, in the therapy, research and prevention of allergic respiratory diseases) (1, 2).
To achieve these goals, however, all sampling points should be placed on the basis of criteria of maximum spatial representativeness that provide a constant flow of atmospheric pollution data and accurately describe the contribution of the main emission Taxa around monitoring stations.
Whereas, for air quality, the European Directives require that AQ stations are classified by type of area and type of station, on the other side, the European Standard for pollen networks, doesn't provide anything but instructions on the micro-localization of the samplers and nothing is indicated on the characterization of the sites based on the dominant sources.
In the absence of regulatory indication for aerobiological monitoring, to evaluate the pollen concentrations we considered an original and widely applicable method for the classification of pollen monitoring stations based on land use data from the Corine Land Cover inventory, calculating the different land use in a buffer centred on our pollen stations. In this way it is easy to obtain a general classification of the sampling site, comparable to that used at European level for air quality networks (see Figure 1).

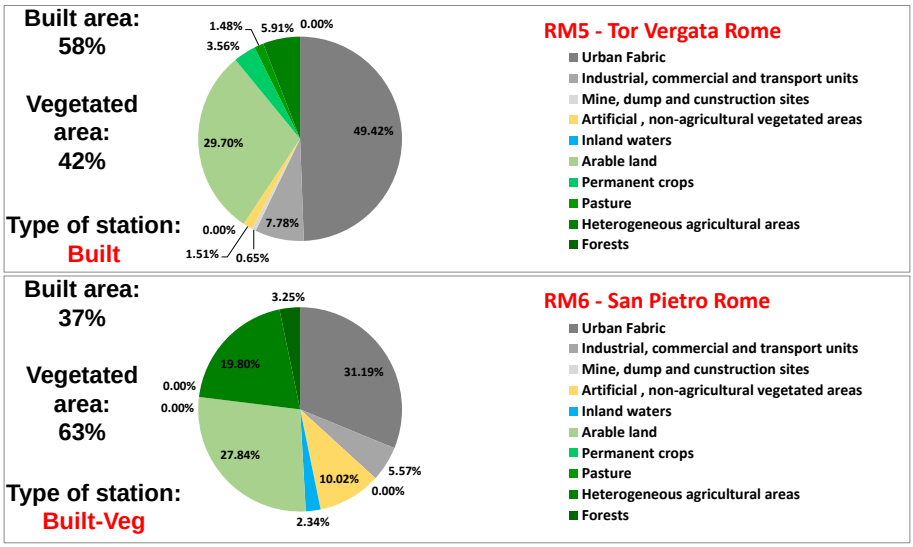


Fig. 1. Classification of two pollen monitoring stations in Rome

1) A. Miraglia, M.A. Brighetti, D. De Franco, A. Di Menno di Bucchianico, F. Froio, A. Travaglini (2023) Sustainability, 15, 4155. https://doi.org/10.3390/su15054155
2) A. Di Menno di Bucchianico, R. Gaddi, M.A. Brighetti, D. De Franco, A. Miraglia, A. Travaglini (2023) Sustainability 15, 6150, https://doi.org/10.3390/su15076150

## 4.8 = Airborne pollution effects on *Tillandsia usneoides* induced by iron oxide nanoparticles

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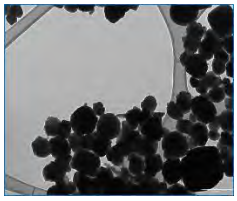
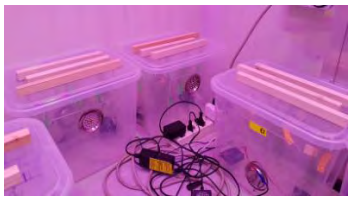
Due to the increasing evidence of widespread nanopollutants in the air, the impact on plants of airborne iron nanoparticles (IONPs) was tested by administering particles with dimensions of 7 nm and 40 nm (Figure 1) respectively, on *Tillandsia usneoides* for two weeks (1). To simulate IONPs spreading in the air, an *ad hoc* designed set-up was constructed as shown in Figure 2. In closed boxes, nine *T. usneoides* strands of about 30 cm in length were exposed to airborne IONPs pollution. Plant samples were folded up and hung to the lid by means of hooks and nanoparticles were resuspended by two fans directed towards the bottom of the box to create a turbulent motion. *T. usneoides* strands were exposed to levels of IONPs reported in the roadside air i.e. in the order of 7 x $10^7$ items $m^{-2}$ and 7x$10^8$ items $m^{-2}$ (2). Here we showed that exposure to IONPs, significantly reduced plant growth with respect to controls depending on the dose. In particular, IONPs at the highest concentration showed the most negative effects on growth in 15 days if compared with the controls. Plants exposed to the concentration 7 x $10^7$ items $m^{-2}$ showed a reduction in growth less than the highest concentration. When present, the IONPs-mediated reduction in plant growth was linked to alterations in the plant concentration of macro- and micronutrients but not to the photosynthetic activity. The IONPs particles were shown to adhere to the plant surface and, preferentially, on the trichome wings. Our results reported, for the first time, evidence of negative effects of airborne IONPs pollution on plant health, thus raising concerns for related environmental risks.





| | Slope | R | P Value |
|---|---|---|---|
| CONTROL | 1,056±0,05492 c | 0,8095 | <0,0001 |
| | | | |
| MNPs 4 $10^7$ items/$m^3$ | 0,7364±0,03325 b | 0,8493 | <0,0001 |
| MNPs 4 $10^8$ items/$m^3$ | 0,5554±0,02777 a | 0,8214 | <0,0001 |
| | | | |
| SNPs 4 $10^7$ items/$m^3$ | 0,8091±0,04928 b | 0,756 | <0,0001 |
| SNPs 4 $10^8$ items/$m^3$ | 0,5574±0,02896 a | 0,8088 | <0,0001 |

Fig. 1. TEM micrographs of IONPs (average dimension 40nm). Fig. 2. Boxes dedicated to airborne pollutant exposure. Fig. 3. Increment in length (cm) of *Tillandsia usneoides* strands exposed to IONPs for 15 days. Letters above the data points of the 9 replicates indicate the significant differences with respect to the control at each single exposure time according to Tukey's test (at least $p < 0.05$).

1) S. Falsini, I. Colzi, D. Chelazzi, M. Dainelli, S. Schiff, A. Papini, A. Coppi, C. Gonnelli, S. Ristori (2022) Journal of Hazardous Materials, 437, 2022, 129314. DOI: https://doi.org/10.1016/j.jhazmat.2022.129314
2) B.A. Mahera, I.A.M. Ahmed, V. Karloukovski, D.A. MacLaren, P.G. Foulds, D. Allsop, D.M.A. Mann, R. Torres-Jardón, L. Calderon-Garciduenasg (2016) PNAS, 113(39), 10797-10801.

## 4.8 = Divergent patterns of metal/metalloid responsiveness and detoxification of two *Medicago truncatula* phytochelatin synthase genes

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Phytochelatin synthase (PCS) is arguably the most important enzyme for the tolerance to toxic concentrations of non-essential metals like cadmium (Cd), lead (Pb) and mercury (Hg) and the metalloid arsenic (As) in angiosperms, thus playing a pivotal role in regulating the accumulation of this toxic ions in the food chain. The low molecular weight oligopeptides synthesized non-ribosomally by PCS from glutathione (GSH), called phytochelatins (PCn), chelate toxic divalent metal cations and trivalent arsenic in the cytoplasm and mediate their sequestration in the vacuole thanks to the action of tonoplast-specific ABCC-type transporters. Despite this pivotal function in the detoxification of toxic metal(loids), overexpression of either heterologous or native *PCS* in a range of different angiosperm species has often been demonstrated to cause hypersensitive phenotypes. For this reason, in most diploid angiosperm genomes studied so far, *PCS* genes are present in one or two constitutively expressed copies. The biosynthetic activity of PCS enzymes has been shown to be induced by a multiplicity of the metal and metalloid ions and comparative studies indicated that different PCS copies can differ in their activation specificity towards these elements.

In this study, we cloned the full length CDS of the two *PCS* genes (*MtPCS1* and *MtPCS2*) present in the fully sequenced genome of the model legume *Medicago truncatula* Gaertn. to compare both *in vitro* and *in vivo* their responsiveness to different essential and non-essential divalent metallic cations and to arsenic. The activity of histidine-tagged recombinant proteins purified from *Escherichia coli* was measured in the presence of two essential (copper, $Cu^{2+}$, and zinc, $Zn^{2+}$) and two non-essential elements ($Cd^{2+}$ and $As^{3+}$). The activity of both copies of MtPCS were stimulated by all the ions tested, and MtPCS2 was in all cases more active than MtPCS1. While the difference in activity in presence of Cu was very similar between MtPCS1 and MtPC2, all other ions cause a much higher activation of MtPCS2 compared to MtPCS1. In line with these results, also *in vivo* the two copies showed different activities in response to these ions: When used for complementing the PCS-deficient *cad1-3* mutant of *Arabidopsis thaliana* L., only *MtPCS2* fully complemented the Cd and As sensitivity to levels comparable to WT, while both *PCS* copies partially complemented Cu sensitivity and none of two PCS copies complemented Zn sensitivity in the *cad1-3* mutant. Quantification of the GSH and PCn pools in the same conditions revealed that Zn and to lesser extent Cd were the ions causing the largest variation of the GSH pool in the shoots, while Cd had the largest effect on the induction of PCn production.

Like *in vitro*, MtPCS2 tends to have higher activity than MtPCS1 either constitutively or in the presence of Cd and MtPCS2 was also highly responsive to Cu. By contrast in the roots, in transgenic lines of both *MtPCS* copies Cd increased the GSH pool, while only in the *MtPCS2* overexpressors As decreased it. Cd had the largest effect on the induction of PCn production in the roots, with MtPCS2 having a higher activity than MtPCS1. Overexpression of MtPCS2 but not of MtPCS1 led to a high accumulation of Cd in both shoots and roots and to a lesser extent of As in the roots.

Taken together, these results, suggest a significant sub-functionalization of MtPCS1 compared to MtPCS2. Additionally, as reported for all *PCS* genes functionally characterized until now from both tracheophytes and bryophytes (1), Cd is the strongest inducer of both MtPCS copies activity both *in vitro* and *in vivo*, suggesting that detoxification of this highly toxic metal is an important function of PCS in extant land plants.

1) M. Li, E. Barbaro, E. Bellini, A. Saba, L. Sanità di Toppi, C. Varotto (2020) Journal of Experimental Botany 71(20): 6655–69. https://doi.org/10.1093/jxb/eraa386

## 4.8 = Soil microbial diversity in urban parks under two vegetation covers

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Soil is a fundamental component for supporting the life on Earth and a main reservoir of biodiversity. Soil biota contribute to several ecosystem services including soil fertility, biogeochemical cycles of nutrients and plant health and productivity. However, anthropogenic activities, including urbanization, can affect soil biodiversity and, indirectly, plant health (1, 2). Within the National Biodiversity Future Centre (NBFC) project we aim to investigate the taxonomic and functional biodiversity of urban soils focusing on the microscopic component (virus, prokaryotes and eukaryotes) and assess their relationships with plant coverage and physico-chemical soil features with the long-term aim to support the design of urban greenspaces more resilient to climate changes as well as restoration programs.
In autumn 2022, a pilot experiment has been set up considering different public parks in the city of Turin. We sampled soils under two different plant coverage (tree and grassland) from three historical parks, five recently established parks (established after 2000) and from two natural/undisturbed environments. A shotgun metagenomics approach was performed on 61 samples using Illumina sequencing. At first, reads were assigned with Kaiju to profile Virus, Prokaryotes, Fungi and microbial Eukaryotes community diversity and structure. Results showed that the most represented phylum are organic decomposers, including Actinomycetota (Prokaryotes), Ascomycota and Basidomycota (Fungi) (Fig. 1). Microbial Eukaryotes diversity appeared more uniform being mainly composed of photosynthetic algae (Chlorophyta) and protist predators such as Evosea. In addition, more than 75% of the detected viruses belong to Uroviricota which includes the widespread dsDNA phages. Moreover, alpha index showed a greater diversity for Prokaryotes and Fungi (Fig. 2), and lesser diversity for other Eukaryotes, in urban parks compared to native undisturbed environments. Finally, beta-diversity analysis revealed a significant impact of the age of the park and the soil cover type on nearly all the four communities studied. As an example, for Fungi the park age explained more than 6% of variance whereas vegetation accounted for 9%. The assembly and annotation of the sequenced metagenomes is still ongoing: results will shed the light on metabolic pathways and biological processes which characterize urban soils compared to native ecosystems. This knowledge will contribute to the improvement of ecosystem services provided by urban green spaces and the success of re-forestation projects towards an enhancement of the quality of life of citizens.

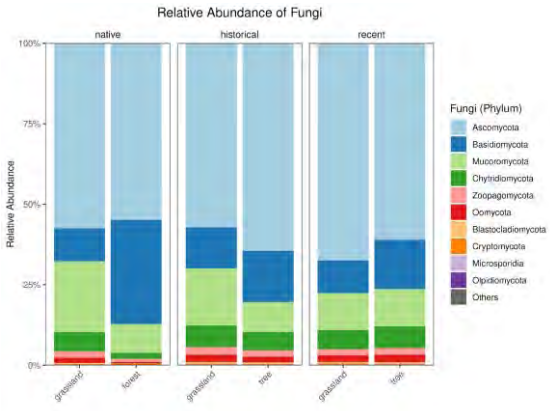


Fig. 1. Relative abundance of Fungi in different parks and under different vegetation type.

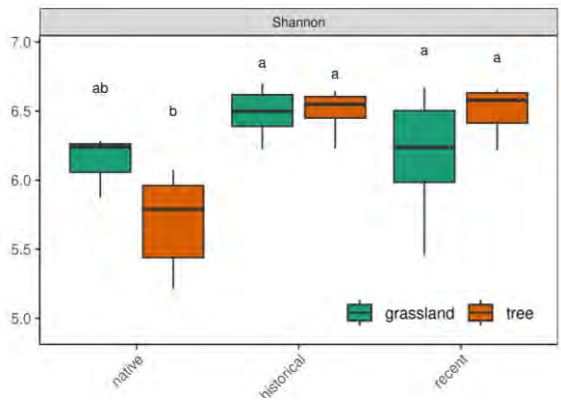


Fig. 2. Shannon index of Fungi in different parks and under different vegetation type.

1) K. Fan, H. Chu, D.J. Eldridge, J.J. Gaitan, Y. Liu, B. Sokoya, J. Wang, H. Hu, J. He, W. Sun, H. Cui, F. D. Alfaro, S. Abades, F. Bastida, M. Díaz-López, A.R. Bamigboye, M. Berdugo, J.L. Blanco-Pastor, T. Grebenc, J. Duran, J.G. Illán, T.P. Makhalanyane, A. Mukherjee, T.U. Nahberger, G.F. Peñaloza-Bojacá, C. Plaza, J.P. Verma, A. Rey, A. Rodríguez, C. Siebe, A.L. Teixido, P. Trivedi, L. Wang, J. Wang, T. Yang, X. Zhou, X. Zhou, E. Zaady, L. Tedersoo, M. Delgado-Baquerizo (2023) Nat Ecol Evol 7, 113–126.
2) S. Geisen, D.H. Wall, W.H. van der Putten (2019) Current Biology, 29, R1036–R1044.

## 4.8 = Biodiversity and ecophysiology of vicariant lichens on limestone and serpentine outcrops in Tuscany

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This study is part of the project "Ecophysiology of Mediterranean dwelling lichens in response to abiotic stresses, with a focus on the genus *Solenopsora*", which aims to investigate lichen diversity on serpentinite and limestone outcrops, as well as photosynthetic performances and elements uptake using selected members of the lichen genus *Solenopsora* as a model.
The study focuses on two species with placodioid thallus, the widespread *S. candicans* (growing on calcareous rocks) and its vicariant *S. liparina* (confined to ultramafic substrates). In particular, the presence of serpentine (and other types of ultramafic outcrops) limits the occurrence of *S. liparina*, which is so far known only in a few sites in Italy, though frequently especially along the Tyrrhenian side.
Thirty-six plots were selected in Southern Tuscany, being equally distributed over serpentinite outcrops (with and without the presence of *S. liparina*) and limestone outcrops (with and without the presence of *S. candicans*). For each plot, the overall lichen diversity (frequency and cover) was surveyed using a square grid (50×50 $cm^2$) divided into 25 quadrats placed on randomly the selected rocks. In addition, photobiont responses of both *S. liparina* and *S. candicans* were assessed by means of chlorophyll *a* fluorescence emission analysis (by a Plant Efficiency Analyzer - Handy PEA), to test the effects of different light and water regimes under controlled conditions, linking their responses to the features of their native environments.

## 4.8 = How to safeguard grapevine autochthonous species from climate change: investigating the resistance to embolism in four cultivars from the Campania region

Francesca Petracca[1], Sylvain Delzon[2], Déborah Corso[2], Camille Parise[2], Chloe Delmas[3], Arturo Erbaggio[4], Chiara Cirillo[1], Veronica De Micco[1]
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The cultivation of grapevine has an important economic and cultural impact worldwide. With rising temperatures and less frequent rainfall, some regions are becoming drier and drier, and some vineyards could be seriously affected or even threatened. Mild stress conditions can improve the quality of red berried grapes, but severe stress can have serious negative effects on plant physiology with consequent reduction of yield and fruit quality (1). Failure of the water transport reduces the efficiency of photosynthesis and therefore productivity. During water stress, air bubbles form and spread within the vascular system (embolism), leading to partial or complete desiccation of the organ or the entire plant. There is therefore an urgent need to better understand the mechanisms by which grapes cope with drought, especially varieties grown in rainfed conditions for the production of wines with quality labels.
The aim of this study was to characterize the xylem vulnerability to embolism of four autochthonous Campanian cultivars of *Vitis vinifera* subsp. *vinifera*: 'Greco', 'Fiano', 'Falanghina' and 'Aglianico'. The plants, grafted on the 420A rootstock, were grown under the same fertigation and climatic conditions in a greenhouse at INRAE for two years. The analyses were performed using two different techniques. The optical technique was performed on expanded leaves of 1-year-old intact vines to visualize the spread of embolism through the leaf vein network by the use of scanners (2). The centrifugal flow technique was performed on two-year-old vine stems using a 100 cm diameter rotor (CAVI1000) allowing the measurement of the Percentage Loss of hydraulic Conductivity (PLC) and therefore the xylem vulnerability to embolism of each plant (3). Results showed that the four cultivars are characterized by different levels of xylem vulnerability to embolism affecting their plasticity in response to cultivation and environmental factors. Gained data will be further analyzed together with leaf and wood anatomical traits to highlight possible coordination between stem and leaf eco-physiological and anatomical traits.

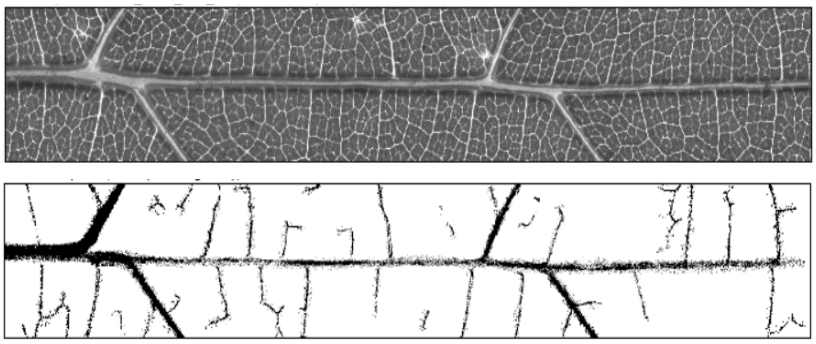

Fig. 1. Scanned image of *Vitis vinifera* subsp. *vinifera* and cumulative image of embolism events recorded on the leaf.

1) M.M. Chaves, O. Zarrouk, J.M. Francisco Costa, T. Santos, A.P. Regalado, M.L. Rodrigues, C.M. Lopes. (2010) Ann. Bot., 105, 661-676.
2) L.J. Lamarque, C.E. Delmas, G. Charrier, R. Burlett, N. Dell'Acqua, J. Pouzoulet, G.A. Gambetta, S. Delzon (2023) Scientific Reports, 13(1), 7724.
3) R. Burlett, C. Parise, G. Capdeville, H. Cochard, L.J. Lamarque, A. King, S. Delzon (2022) Annals of Forest Science, 79(1), 1-16.

## 4.8 = Due macro and microplastics affect rhizosphere and plant health? The case study of *Lamium flexuosum*

Maria Antonietta Ranauda[1], Maria Maisto[1], Maria Tartaglia[1], Daniela Zuzolo[1], Alessia Postiglione[1], Antonello Prigioniero[1], Pierpaolo Scarano[1], Alessandra Falzarano[1], Rosaria Sciarrillo[1], Carmine Guarino[1]
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Pollution by micro- and macroplastics (MPs) has a significant ecological impact on a wide variety of ecosystems. Research on this emerging contaminant has increased considerably in recent years, but little is known about its effects on plants. The rhizospheric microbial community plays a crucial role in plant fitness maintenance, so it is essential to understand how the plant-microbiome root meta-organism interacts in response to environmental changes such as the soil contaminant occurrence. This work investigated the effects of oxidised high-density polyethylene (HDPE) on *Lamium flexuosum* Ten. (Lamiaceae). In detail, this study analysed the complex microbial dynamics at the rhizospheric level and how MPs contamination in soil can influence them. The metagenomic approach provided an overall assessment of the microbial activity in the rhizospheric soil; in addition, the comparison with metagenomic analyses of uncontaminated soil allowed to highlight the taxonomic shift induced by plastics presence. To assess the physiological plant state, the activities of the main enzymes related to oxidative stress (ascorbate peroxidase, glutathione reductase, catalase, superoxide dismutase) and photosynthetic pigments (chlorophylls, carotenoids) were analysed. Also the colonisation at root level by arbuscular mycorrhizal fungi (AMF) was evaluated. Finally, the plastisphere was studied both by metagenomic approach and by biofilm observations at the scanning electron microscope (SEM) with the idea that different types of biofilms could affect soil ecology and, consequently, might influence plant behaviour. The results suggest that the presence of MPs in the soil causes changes in the soil and rhizosphere microbial community and this could also have negative effects on plants, which showed a significant decrease in chlorophyll levels and variations in the level of enzymes involved in oxidative stress. The antioxidant defence system seems to play a protective role in plants grown in MPs contaminated soil. The rate of AMF roots colonisation also showed differences in plants exposed to this environmental pollutant compared to plants grown in uncontaminated soil. Finally, MPs SEM observations revealed the presence of a complex colonisation characterized by stick-shaped, discoidal and spheroidal cells, fungal hyphae and exopolysaccharide substances (EPS) constituting a particular ecological niche that could affect the surrounding environment.

## 4.8 = Effectiveness on nature-based solutions in the city of Genoa

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Nature-based Solutions (NbS) are actions to protect, sustainably manage or restore natural ecosystems (1). Although ecosystem services are well-known (2), a clear assessment of NbS effectiveness with quantitative key performance indicators (KPIs) has not been yet completely standardized (3).
At the former Gavoglio military barracks (Genoa, NW Italy), the project H2020 UnaLab allowed setting up restoration practices, during 2020-2022, to shape an urban park of 13 ha with 12 different NbS for stormwater management whose benefits should be assessed.
The aim of the study was to assess the NbS through KPIs. The biodiversity (plants, pollinators, and birds) has been evaluated through the Shannon's index, birds' species richness, ratio between passerine and non-passerine species and related multitrophic interactions; the evapotranspiration rate was calculated through Thornthwaite's equation with on-site measurements with weather station, and the carbon storage assessed via evaluation of the woody biomass and $CO_2$ equivalent stored.
All the evaluated KPIs highlighted a low-biodiversity condition with 52 plant *taxa*, 25 pollinator insect species and 26 bird species. This is probably related to the time required to restore the urban area that required more time to allow a good colonization by stable population of animals and to overcome the planting stress. A subsequent monitoring with the same KPIs will provide additional data to evaluate changes in all the selected KPIs.
The study results and the comparison with the other front-runner cities involved in the Unalab project will enable the development of a European NBS Reference Framework on benefits, and replicability of NbS, which will guide cities across Europe and beyond.

1) E. Cohen-Shacham, G. Walters, C. Janzen, S. Maginnis (2016) Nature Based Solutions to address global societal challenges. IUCN, ISBN 978-2-8317-1812-5.
2) J. Maes, A. Teller, M. Erhard et al. (2020) Mapping and Assessment of Ecosystems and their Services: An EU ecosystem assessment, EUR 30161 EN, Publications Office of the European Union, Luxembourg, ISBN 978-92-76-17833-0, doi:10.2760/757183, JRC120383.
3) A. Andrade, E. Cohen-Shacham, J. Dalton, S. Edwards, D. Hessenberger, S. Maginnis, S. Maynard, P. McElwee, R. Murti, C. Nelson, V. Ruiz, J. Siikamäki, L. Vasseur (2020) Guidance for using the IUCN Global Standard for Nature-based Solutions: first edition. 10.2305/IUCN.CH.2020.09.en

## 4.8 = Different size of Zn Oxide particles may affect root development in *Oryza sativa*

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Nanotechnologies are a tremendous opportunity for their positive impact in many sectors of industry and scientific research, but one major drawback is the release of nanometer-sized materials into aquatic, terrestrial and atmosphere environments, so much so that they are considered emerging contaminants of particular concern.
ZnO nanoparticles are among the top five nanoparticles (NPs) used in a wide range of applications: in the production of paints, coatings, inks, plastics, cosmetics and personal care products, among the others, with an annual production that has reached 8,000 tons (1). This high production and the wide use of NP-ZnO-containing products obviously increase the probability of leakage into the environment with growing risks for living organisms (2). It is therefore crucial to produce further scientific data in different disciplines to deeply understand some of the possible environmental hazards associated with ZnO NPs. Higher plants, tightly interacting with their environment, are good test organisms to assess in depth NPs impact on ecosystems and food chains.
A large number of literature data indicate that ZnO NPs are able to affect plant growth and root development/architecture, with damage/shortening of the root tip in NP-ZnO-treated ryegrass (3) and alteration of root morphology in wheat under treatment with both NP-ZnO and the bulk counterpart (2).
It has been suggested that this negative impact could be due to the influence of Zn on the homeostasis of hormones, particularly auxin (IAA), which is crucial for the regulation of root growth and lateral roots (LR) development. To assess this hypothesis, caryopses of the model plant *Oryza sativa* L., were treated with two different concentrations of ZnO NPs and of their bulk counterpart, as another aim was to evaluate if ZnO action could depend on particle size. Besides root growth and development assessment, oxidative stress parameters, determined both biochemically and histochemically, IAA content and molecules/enzymes involved in IAA metabolism were analysed. The *in situ* localization of Zn in control and treated roots was also performed. Though only NPs were visible inside root cells, both materials were able to affect seedling growth and root morphology, with alteration in the concentration/pattern of localization of oxidative stress markers, and with a different action depending on particle size. In addition, only ZnO supplied as bulk material induced a significant increase both in IAA concentration and in LR density, supporting the hypothesis that the particles of this size might enhance LR development through the rise of IAA concentration, which appeared to be influenced more by the activity of the catabolic peroxidases than by the protective action of phenols. While the effects induced by NP-ZnO could be attributed to a direct interaction with plant cells, the larger size of B-ZnO particles probably prevents them from overcoming plant cell barriers, and their action might be indirect, due to adhesion to root surface.

1) L. Vimercati, D. Cavone, A. Caputi, L. De Maria, M. Tria, E. Prato, G.M. Ferri (2020) Front. Public Health, 8, 192.
2) C. Spanò, S. Bottega, C. Sorce, G. Bartoli, M. Ruffini Castiglione (2019) Environ. Sci. Pollut. Res., 26, 29872–29882.
3) D. Lin, B. Xing (2008) Environ. Sci. Technol., 42, 5580-5585.

## 4.8 = Twenty years of pollen monitoring activity at Rome Tor Vergata (Rome south-east): trends analysis

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Aerobiological pollen monitoring is a potential tool to reveal the biological response of plants to changing climate. Altering the dynamics of terrestrial vegetation has consequences for the functioning of the Earth system and risks for human and natural systems; for example, there is an increase in cases of pollinosis and asthma (1). To fully exploit the potential of airborne pollen pollen as a bio-indicator of climate change it is it is necessary to establish the relationships between weather-climate variables and territory.
In light of this, meteorological variables (temperature and precipitation) and pollen trends obtained from aerobiological monitoring data from the Tor Vergata station in Rome, in the period 1997-2016, were analysed using the standardised methodology according to CEN/TS 16868:2019.
With regard to meteorological variables, a significant increase in summer temperatures is noted.
The analysis of the pollen spectra of Rome (Fig.1) shows a statistically significant growth trend for the two herbaceous taxa, Plantaginaceae and Urticaceae, characterised by species that show a high capacity to live under stressful conditions with high levels of nitrogen in the soil and at high temperatures, probably due to the increase in the urban fabric and the gradual abandonment of agricultural practices in the surrounding area; and a decrease in flowering intensity in the Amaranthaceae.
Regarding the flowering season, only for the Poaceae does the start date show a statistically significant advance, evidence in line with recent investigations of long-term aerobiological datasets in long-term aerobiological datasets in Europe that indicate an earlier onset of the pollen season.
earlier onset of pollen seasons (2), which in the case of Rome cannot be attributed to increased summer temperatures.

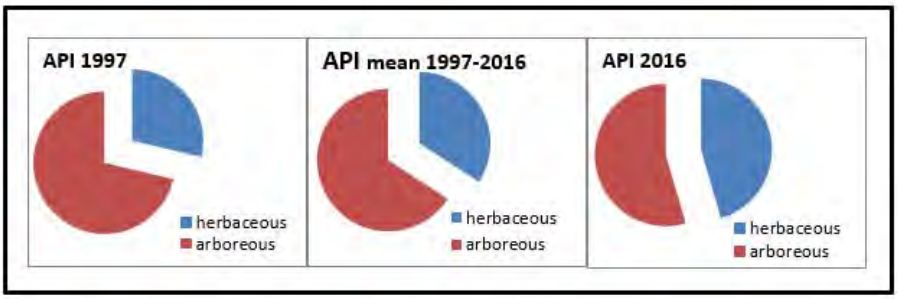


Fig. 1. Pollen spectrum in Rome.

1) C. Ziello, T.H. Sparks, N. Estrella, J. Belmonte, K.C. Bergmann, E. Bucher, M.A. Brighetti, A. Damialis, M. Detandt, C. Galán, R. Gehrig, L. Grewling, A.M. Gutierrez Bustillo, M. Hallsdottir, M.C. Kockhans-Bieda, C. De Linares, D. Myszkowska, A. Paldy, A., Sanchez, M. Smith, M. Thibaudon, A. Travaglini, A. Uruska, R.M. Valencia-Barrera, D. Vokou, R. Wachter, L.A. De Weger, A. Menzel (2012) Plos One, 7, e34076, https://doi: 10.1371/journal.pone.003407
2) A. Di Menno di Bucchianico, R. Gaddi, M.A. Brighetti, D. De Franco, A. Miraglia, A. Travaglini (2023) Sustainability, 15, 6150, https://doi.org/10.3390/su15076150

## 5.1 = Phenotyping of several olive plants from the CREA OFA olive collection field based on vessel occlusion and metabolomic analysis

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Progression of the *Xylella fastidiosa* infection suggests it has the potential to break through current barriers in Puglia and extend to large areas of Italy and Mediterranean counties. The olive tree appears to be the most sensitive crop, with a higher incidence on trees older than 30 years. The bacterium determines a symptomatology called olive quick decline syndrome (OQDS) (1), characterized by desiccation of variable portions of the foliage, leading in many cases to the death of the host plant. Some varieties such as Ogliarola salentina showed to be more susceptible than others such as Leccino.
Olive growing in Puglia, but also in Italy, urgently needs to select olive germplasm tolerant/resistant to *Xylella fastidiosa* subsp. *pauca* ST 53 but experimenting with the pathogen is a limiting factor and both phytopathology progression and varietal selection are long processes.
The identification of phenotypic traits predictive of disease progression may represent an innovation in the search for tolerant olive tree varieties.
Investigation on the vessel occlusion mechanism provided an insight into the plant response to bacterial colonization of xylem (2; 3) and we postulate it can be and efficient parameter to be used as varietal selection marker.
Occlusions, in any of the multiple forms realized in the plant xylem, represent the reaction of the plant to biotic stress. We analysed the section of 2 years old branches from 20 varieties of *O. europaea* cultivated in the field collection of Mirto Crosia (Cosenza province, Italy) evaluating the occurrence of natural occlusions in the absence of evident phytopathology.
The initial results suggested that each variety can be characterized by a specific frequency of occlusions. The metabolome of the same varieties was then analysed searching for metabolites associated to the newly defined trait. Although the study is currently ongoing, the histological trait of xylematic occlusions, combined with the metabolic profile, appear a promising varietal selection marker.

1) M. Saponari et al. (2019) Phytopathology, 109(2), 175-186. https://doi.org/10.1094/PHYTO-08-18-0319-FI
2) M. De Benedictis et al. (2017) Journal of Phytopathology, 165(9), 589–594. https://doi.org/10.1111/jph.12596
3) E. Sabella et al. (2019) Sci Rep 9, 9602. https://doi.org/10.1038/s41598-019-46092-0

## 5.1 = Study of pollinator and plant communities in agroecosystems in Emilia-Romagna, Italy

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In the last fifty years, an alarming decline in pollinator populations has been observed worldwide[1]. Insect-mediated pollination is of fundamental importance for both natural and agricultural ecosystems[2]. Therefore, the implementation of pollinator-friendly actions in agroecosystems, such as flower strips, artificial nesting sites and non-agrochemical pest management, is urgently needed[3]. At the national level, landowners can support the safeguard of wild pollinators through funding from the Strategic Plan of the Italian CAP (Common Agricultural Policy). Within the LIFE 4 Pollinators project (LIFE18 GIE/IT/000755) we tested the effectiveness of the above-mentioned measures through an ex-ante and ex-post monitoring in 12 farms in Emilia-Romagna, Italy. Here we present the preliminary results of the ex-ante phase (2022), that is prior to the implementation of the "pollinator-friendly" actions by the landowners. The trend and presence of wild pollinators is surveyed using a mobile transect following the seasonal blooms, from April to August (Fig.1). Flower visitors are recorded and determined at genus level (bees) or broader taxonomic level (Fig.2). Floral resource availability within each transect and in selected plots is also assessed (Fig.3). To investigate the contextual characteristics of the study areas, we also collected the use of soil in and around farmlands. Our findings indicate that the diversity of pollinators and their interactions with entomophilous plants are strongly affected by agronomic management, land use in nearby areas, and floral resource availability and variety. In particular, conventional farms showed lower pollinator abundance, as well as flowering plant and bee diversity. We therefore expect an environmental benefit as a result of pollinator-friendly measures in the farmland.

Fig. 1. Transect and plots within study site.

Fig. 2. Pollinator samples.

Fig. 3. Floral resource assessment.

1) E.E. Zattara, M.A. Aizen (2021) One Earth, 4(1), 114–123.
2) D.M. Katumo, H. Liang, A.C. Ochola, M. Lv, Q. Wang, C. Yang (2022) Plant Diversity, 44(5), 429–435.
3) P.A. Henríquez-Piskulich, C. Schapheer, N.J. Vereecken, C.A. Villagra (2021) Sustainability, 13(12), 6728.

## 5.1 = Stomatal and mesophyll limitations to photosynthesis in contrasting *Triticum* varieties

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The most recent Intergovernmental Panel on Climate Change report provides a range of scenarios for future $CO_2$ concentrations, depending on assumptions about future greenhouse gas emissions (IPCC, 2021). Without mitigation actions, under the most optimistic scenario, atmospheric $CO_2$ concentrations would reach 420 ppm by the end of the century, which is slightly above the highest levels recorded during the middle Pliocene, about 3 million years ago. Under the more pessimistic scenario, atmospheric $CO_2$ concentrations would reach 1100 ppm by the end of the century [1]. Therefore, it is essential to understand the impact of rising $CO_2$ on plant physiology and growth for the sustainability of ecosystems, agriculture, and food security. This is the background of the Evolutionary implications for the development of climate resilient productive plants PRIN project (EvoPlant), which aims to elucidate patterns of plant evolution to promote the development of climate-resilient plants through the study of stomatal evolution, photosynthesis, and plant-atmosphere gas exchange. In plant evolutionary studies there are clear evolutionary trends towards increased conductance to $CO_2$, biochemical efficiency of photosynthesis, water transport, and stomatal control across basal groups to more recently derived angiosperms. A special case is represented by *Triticum* spp., the ancestral neglected varieties of wheat are considered to be more climate resilient than the more productive modern cultivars. Traditional breeding methods may have favored productivity over stress resistance. One possible cause of this loss of climate resilience may be the increase in genome size in modern wheat cultivars. Stomatal dimensions are positively related to genome size [2]. Large stomata are considered less likely to respond rapidly to environmental stimuli than their smaller counterparts due to the larger volume to surface area ratio of guard cells in large stomatal complexes [3]. Our aim is to study the transport of $CO_2$ across the mesophyll within the *Triticum* spp. from ancestral to modern varieties using the leaf anatomical method. One of the main physiological processes limiting $CO_2$ uptake and fixation during photosynthesis is mesophyll conductance ($G_m$). Mesophyll conductance quantifies the diffusion of $CO_2$ from intercellular airspace within a leaf to the sites of Rubisco carboxylation within chloroplasts. We are currently completing morphological and anatomical analyses of the *Triticum* evolutionary transept to calculate the $G_m$ and the diffusion coefficient to assess how individual leaf anatomical trait limit $CO_2$ uptake and fixation during photosynthesis in the different *Triticum* genotypes.

1) IPCC (2021) Summary for Policymakers. In: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 3−32.
2) J.M. Beaulieu et al. (2008) New Phytologist, 179(4), 975-986.
3) J.A. Raven (2014) Journal of Experimental Botany, 65(6), 1415-1424.

## 5.1 = Restoring agro-ecosystems by reintroducing rare arable species: competitive effects on species growth and crop productivity

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Arable species include the set of wild plants present in agro-ecosystems that are well adapted to disturbed environments and that evolved with different agricultural practices (1). After 1950, due to the rapid intensification of agriculture, arable species undergone a dramatic decline, especially those closely linked to traditional agricultural practices. In most cases arable species are considered as weeds, as their natural habitat is found within crop fields and for this reason, they are systematically eradicated in conventional farming systems. Although sustainable agricultural systems such as organic and low-input farming systems are more widespread than ever, arable species extirpated even in these systems are no longer able to recolonize their former range. Therefore, the reintroduction of arable species within agro-ecosystems has been proposed to improve their conservation status, as these plants are important components of the agro-ecosystem and may provide ecosystem services (2). Unfortunately, farmers are often reluctant to accept the reintroduction of endangered plants to their lands, as arable species are perceived by farmers as noxious, as they could reduce crop productivity through competition (3).
For this reason, to verify the feasibility of using and conserving rare and declining species for restoring agro-ecosystems we investigated mutual competitive effects between one iconic arable species, *Agrostemma githago* L., that have shown a very strong population decline in recent decades due to the use of non-selective herbicides and changed agricultural practices, and *Triticum aestivum* L. (Palesio variety) in an open field experiment at the SemeNostrum company (Udine, Italy).
We analysed the growth of both species and assessed the impact of the arable species on crop productivity. Analysing the root/shoot ratio, *A. githago*, in competition with the crop, reduces considerably, and clearly the aerial part decreases much more than the root part. This may be related to a problem of light availability and consequently photosynthetic efficiency, which is reflected in the biomass (Fig. 1). For *T. aestivum*, instead, there is no effect on the root/shoot ratio due to the presence of Agrostemma, but only a weak effect of fertilization (Fig. 2). Wheat productivity was not affected by fertilisation or non-fertilisation in the absence of competition, but the difference exists, in terms of grams, in the presence of *A. githago* and is greater the denser the *A. githago* (Fig. 3).
From this first comparative analysis between *A. githago* and *T. aestivum*, it was possible to take a step forward towards the knowledge on the real competitiveness between segetal species and crops, with the future aim of reintroducing extinct or endangered species in agricultural contexts to restore agro-ecosystems.

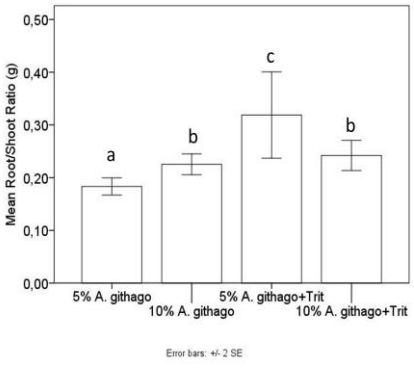


Fig. 1. Effects of *T. aestivum* competition on the root/shoot ratio of *A. githago.*

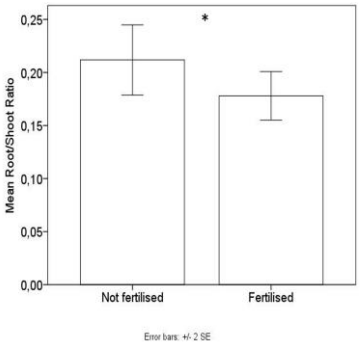


Fig. 2. Effects of *A. githago* competition on the root/shoot ratio of *T. aestivum* with and without fertiliser use.

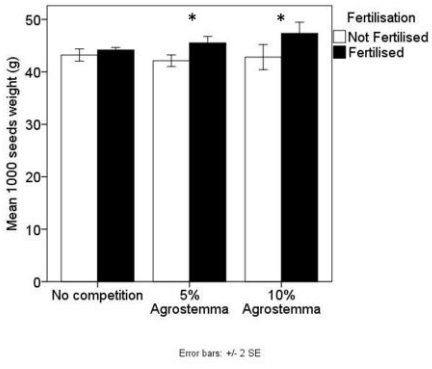


Fig. 3. Effects of *A. githago* competition on the productivity of *T. aestivum.*

1) S. Gaba, R. Perronne, G. Fried, A. Gardarin, F. Bretagnolle, L. Biju-Duval, ... & X. Reboud (2017) Weed Research, 57, 123-147.
2) M. Lang, J. Kollmann, J. Prestele, K. Wiesinger, H. Albrecht (2021) Agriculture, Ecosystems & Environment, 306, 107187.
3) S. Cordeau, X. Reboud, B. Chauvel (2011) Agronomy for sustainable development, 31, 463-473.

## 5.1 = How to detect viral disease in grapevines with two techniques: ELISA and RT-PCR

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The viral diseases that can affect grapevines are numerous and growing. The spread of many of these diseases is uncontrolled and in most cases harmful to plants and consequently to wine production. Of particular interest are some diseases related to the presence of animals, specifically nematode vectors of viruses, capable of infesting the rhizosphere of grapevine. Despite measures to control nematode associated viruses spread, this disease remains problematic in vineyards where it is established.
Hence, it is of interest to monitor the occurrence and spread of viral infection in vineyards. Here we investigated one of the biggest and representative wine-growing region of Southern Italy (Sannio district).
Two diagnostic approaches have been used to ascertain or avert the presence of these pests and their viruses: ELISA and RT-PCR. The need to use two different techniques stems from the fact that, especially at certain times of the year, the concentration of viruses that can be detected by techniques such as ELISA is not sufficient. However, a proper execution of RT-PCR is not always easy, which greatly reduces the reliability of this technique.
Through comparison with these two different techniques, we investigated 8 different phloem viruses that include GLRaV-1, GLRaV-2, GLRaV-3, GFLV, ArMV, GVA, GVB, and GFkV. Each virus has its symptomatology, morphology, and epidemiology. The samples examined to conduct this study consisted of shoots and leaves taken during different times of the year, which were stored at -80°. The results obtained allowed us to detect several viruses in some plant samples, which worsens the phytopathological outline.
The information collected can be used to monitor diseases spread in vineyard and make informed vine management decisions. The monitoring program is ongoing and have to be compiled with those of other vineyards in the area to give a regional picture.

## 5.1 = Conservation of the germplasm of *Capsicum annum* traditional horticultural varieties from Campania Region (Italy)

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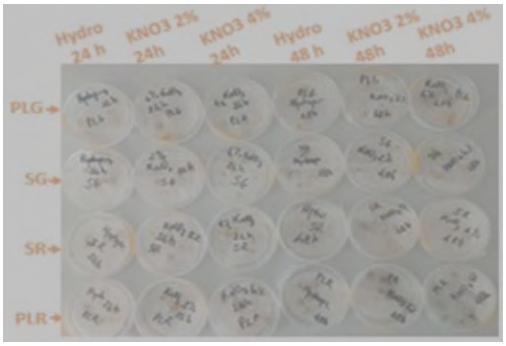
Campania hosts a conspicuous patrimony of traditional horticultural species and varieties; for instance, the tomato, with the *San Marzano, Piennolo, Sorrento* and *Corbarino* cultivars, and some pepper varieties, as *Papaccella*, to name some of the best known.
The conservation of these varieties involves fields of different nature, since they represent a cultural, economic, nutritional, botanical and ecological heritage. The protection of Campania's horticultural biodiversity is of fundamental importance, both in terms of food sources, and in terms of conservation of indigenous plant genetic resources, including entities at risk of extinction. But it is equally important to achieve this objective while respecting the agricultural ecosystem, which must be both productive and sustainable.
The cultivars studied belong to the species *Capsicum annum* L.; in particular, Papaccella Riccia Napoletana (PRN), Papaccella liscia gialla (PLG), Papaccella Liscia Rossa (PLR), Sassaniello Giallo (SG) and Sassaniello Rosso (SR). These varieties show a noticeable decrease of the seed germination percentage after storage at 4 °C for one year. According to all above, several experiments were set up aimed at improving the germination percentage and the plant growth of these pepper varieties.
In a first trial, starting from seeds with and without vernation, collected in fruits at different stages of ripening, we calculated the germination percentage (GP) the TMG (mean time of germination) VI (vigor index). All cultivars needed a 2-months vernation, except PRN. Moreover, the GP was lower in under-ripe fruits, which showed incompletely developed, sometimes non-vital embryos, evidenced by the TTZ test (2,3,5-Triphennyltetrazolium chloride). In a second test we evaluated the effect of different priming methods (hydropriming, acid priming and priming by $KNO_3$) on GP and TMG, as well as on plant development. The first results highlight that hydropriming seems overall the most promising treatment, whereas acid priming induces a significant negative effect in the measured parameters. However, some differences were observed depending on the different cultivar (e.g., hydropriming stimulated plant development in PLG/R, whereas $KNO_3$ in SG/R).
In forthcoming experiments other priming method will be tested (microwave and radiations) parallel to ageing treatment effect, and DNA multilocus analysis to check for the preservation of these cultivars as genetic resources.




1) Buiatti M., Lorenzoni C. (1963) Heinh. Caryologia, 16, 101-110.
2) A. Concellón, M.C. Añón, A.R. Chaves (2007) LWT – Food Science & Technology, 40, 389-396.
3) I.-U.U. Rahman, N. Ali, A. Rab, Z. Shah (2013) Sarhad J. Agric.,29, 379–386.

## 5.1 = Landraces can be resources for the sustainable development of mountain areas: the case of 'Copafam' bean (*Phaseolus coccineus*)

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Nowadays, about 80% of global agrobiodiversity has been lost since the last century (FAO 2004).
Italy is rich in agrobiodiversity but many of traditional cultivars (landraces) are little or not at all scientifically characterized. Landraces are mainly found in hilly and sub-mountain areas (150–800 m a.s.l.) and among the most numerous families there are Fabaceae, together with Poaceae and Solanaceae, able to grow in a wide altitudinal range (from sea level to over 1000 m a.s.l.) (Giupponi et al. 2021). The "Copafam" is a variety of runner scarlet bean *(Phaseolus coccineus* L.) that can be grown only in the mountains. This landrace has a historical importance for italian mountain marginal areas, and it is now at risk of extinction because of depopulation and abandonment of mountain areas. The main objective of this work was the characterization and enhancement of "Copafam" bean in order to evaluate its possible input as a raw material in the agro-food industries for the creation of innovative and functional products. For this purpose, the nutritional and phytochemical characteristics of the "Copafam" bean was explored. Three commercial beans were used as a comparison. Moreover, the sensory properties and consumers' hedonic ratings in a model food formulation (biscuits) made by this landrace was assessed using a citizen science approach. The results show that the "Copafam" bean had a high dietary fiber and protein content (34.83 ± 2.48 g/100 g dw and 21.93 ± 0.41 g/100 g dw) and it resulted in a great source of secondary metabolites as polyphenols (121.36 ± 5.31 mg GAE/g dw), flavonoids (6.51 ± 0.17 mg/kg dw), and anthocyanins (28.11 ± 0.16 mg Cy3 G/kg dw), having remarkable antioxidant activity too (76.42 ± 1.27 %). Specifically, all the beans examined in this study contain similar hydroxycinnamic acid derivatives as their main phenolic component .The samples of *P. coccineus* contained a higher sinapic acid while *P.vulgaris* samples were abundantly composed of ferulic and p-cumaric acid. Furthermore, "Copafam" had the lowest phytic acid content and minor trypsin inhibitory activity (Pedrali et al. 2022).
Biscuits made from "Copafam" flour were considered acceptable by consumers and were characterized by a darker colour, crunchy texture and an uneven appearance compared to the control, probably due to the punctuation detected on the surface of the samples. The high amount of functional molecules found in "Copafam" beans could represent innovative forms of consumption such as fortified foods to enhance agricultural products for human health. This research showed that landraces can represent a great resource for an innovative food industry aiming to preserve agrobiodiversity and promote the sustainable development of mountain areas.

1) FAO (2004) Building on Gender, Agrobiodiversity and Local Knowledge; Food and Agriculture Organization of the United Nations: Rome, Italy.
2) L. Giupponi, D. Pedrali, V. Leoni, A. Rodari, A. Giorgi (2021) Diversity, 13, 70. https://doi.org/10.3390/d13020070
3) D. Pedrali, C. Proserpio, S.M. Borgonovi, M. Zuccolo, V. Leoni, G. Borgonovo, A.M. Bernardi, A. Scarafoni, E. Pagliarini, A. Giorgi, et al. (2022) Sustainability, 14, 13409. https://doi.org/10.3390/su142013409

## 5.1 = Multi-omic characterisation as a tool to improve knowledge, valorisation and conservation of wild fruit genetic resources: the case of *Arbutus unedo*

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The conservation of plant genetic resources (PGRs) is contingent on their current or potential use, so extending the knowledge about them is crucial for their valorization. The valorization and conservation of wild fruit PGRs are critical to ensure the maintenance of genetic and cultural heritage and to promote and develop of new perspectives on resource use. New strategies for characterizing PGRs are needed, and the omics approach can provide profound information that is still largely unknown [1]. The strawberry tree (Arbutus unedo L.) is an underutilized, drought and fire-resistant species distributed in the Mediterranean area and its berries have large ethnobotanical use but were not usually consumed as fresh fruits[2]. Although their phenolic profile and antioxidant capacity are known, they are not well characterised, particularly from a proteomic perspective[3]. The aim of this work is the characterisation from a molecular viewpoint of two ecotypes of A. unedo (Campania and Sicily Fig.1) to valorise a less known wild fruit PGR to encourage its preservation. Samples were collected from two different geographical areas to assess whether different geographical conditions could influence the characteristics of leaves and fruits at the three stages of ripening (green, veraison, red). Proteomic analysis identified 904 proteins, of which 122 showed significances along the ripening process. Some of these differentially abundant proteins, such as chalcone synthase, show a marked increase during ripening. From a proteomic perspective, there are no differences between the fruits from the two regions compared by the ripening stage (Fig.2). However, the pedoclimatic metabolic imprinting allowed the observation of good diversity in the metabolomic profiles between the two ecotypes, especially for anthocyanins and catechins, generally more abundant in Sicilian fruit, or the phenolic compounds more abundant in Campania fruit. Multi-omic characterisation enhanced the knowledge on a wild fruit plant species which shows specific adaptations and responses to the environment to be considered when addressing the issue of local agrobiodiversity.

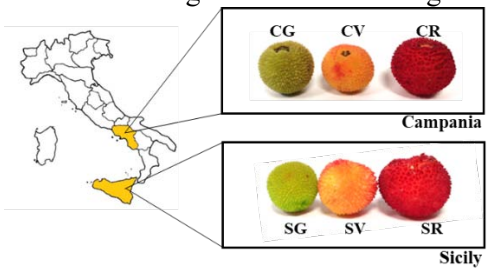


Fig.1. *A.unedo* sampling area and fruits during ripening stages.

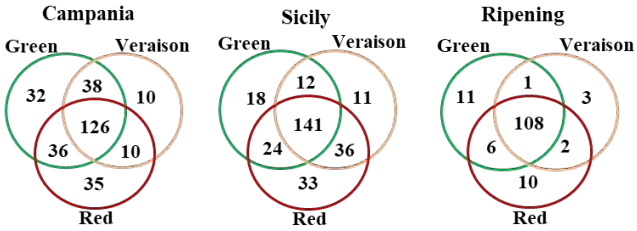


Fig. 2. Venn diagrams showing differential protein expression during ripening stages and according to sampling area.

1) L. Bacchetta, F. Visioli, G. Cappelli, E. Caruso, G. Martin, E. Nemeth, G. Bacchetta, G. Bedini, A. Wezel, T. van Asseldonk, L. van Raamsdonk, F. Mariani J. (2016) Ethnopharmacol., 191, 180–187. https://doi.org/10.1016/j.jep.2016.05.061.

2) B.M. Ruiz-Rodríguez, C. Sánchez-Moreno, B. De Ancos, M. De Cortes Sánchez-Mata, V. Fernández-Ruiz, M. Cámara, J. Tardío (2014) Fruits., 69, 435–448. https://doi.org/10.1051/fruits/2014035.

3) P. Scarano, R. Guida, D. Zuzolo, M. Tartaglia, A. Prigioniero, A. Postiglione, G. Pinto, A. Illiano, A. Amoresano, R. Schicchi, A. Geraci, R. Sciarrillo, C. Guarino (2022) Front. Nutr., 9. https://doi.org/10.3389/fnut.2022.91599.

## 5.1 = The rootstock shape microbial diversity and functionality in rhizosphere of *Vitis vinifera* 'Falanghina'

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The rhizosphere effect occurring at the root-soil interface has increasingly been shown to play a key role in plant fitness and soil functionality, influencing plants resilience (Nadarajah et al., 2023). Here, for the first time, we investigated whether the rootstock genotype on which *Vitis vinifera* L. cultivar Falanghina is grafted can influence the rhizosphere microbiome and potential funcionality. Specifically, we evaluated to which extent the 5BB and 1103P rootstocks are able to shape microbial diversity off rhizosphere environment. Moreover, we explored the potential function of microbial community and its shift under plant genotype influence. We investigated seven vineyards subjected to the same pedo-climatic conditions, similar age, training system and management and collected twelve rhizosphere soil samples for metagenomic analyses and composite soil samples for physical-chemical properties. In this study, we used 16S rRNA gene-based metagenomic analysis to investigate the rhizosphere bacterial diversity and composition. Liner discriminant analysis effect size (LEFSe) analysis was conducted for metagenomic biomarker discovery (Cao et al., 2022). The functional composition of sampled communities was determined by using PICRUSt, which is based on marker gene sequencing profiles (Langille et al., 2013). Soil analyses involved the determination of texture, pH, Cation Exchange Capacity (CSC), Organic Carbon (OC), electrical conductivity (EC), calcium (Ca), magnesium (Mg), potassium (K) content, Phosphorous (P), nitrogen (N). The metagenomic data showed that the bacterial alpha-diversity (Observed OTUs) significantly increased in 1103P rhizosphere microbiota. The latter revealed that soil was quite homogenous. Irrespective of cultivar, Proteobacteria was the dominant phylum, followed by Actinobacteria > Bacteroidetes > Crenarchaeota. Proteobacteria. However, Actinobacteria was the major marker phyla differentiating the rhizosphere microbial communities associated with the different rootstock types. At the genus level, several Actinobacteria and Alphaproteobacteria genera were enriched in 1103P genotype rhizosphere. Investigating the potential functional profile, we found that most key enzyme-encoding genes involved in N cycling were significantly more abundant in 5BB rootstock rhizosphere soil. However, we found that 1103P rhizosphere was enriched in genes involved in C cycle and PGP functionality. Our results suggest that the different rootstocks recruit specific bacterial communities and specific functional traits within the same environment.

1) K. Nadarajah, N.S.N. Abdul Rahman (2023) Plants, 12, 2307. doi:10.3390/plants12122307.
2) Y. Cao, Q. Dong, D. Wang, P. Zhang, Y. Liu, C. Niu (2022) Bioinformatics, 38, 4027–4029. doi: 10.1093/bioinformatics/btac438.
3) M.G.I. Langille, J. Zaneveld, J.G. Caporaso, D. McDonald, D. Knights, J.A. Reyes et al. (2013) Nat. Biotechnol., 31, 814–821. doi: 10.1038/nbt.2676.

## 5.2 = Harnessing plant-bacteria interactions to improve plant health

Giulia Ghirardello[1,2], Nadine De Biasio[1,2], Rosalba Cipriani[1,2], Tiziano Bonato[3], Werner Zanardi[3], Barbara Baldan[1,2], Sebastiano Nigris[1,2]
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Plants cohabit with a huge diversity of microorganisms in the soil (bacteria, fungi, archaea and protists), known as root microbiota (Fig. 1). The microbial communities are key contributors to plant growth and performance by enhancing nutrient acquisition, immunity and tolerance to environmental stresses (1; 2). The use of microorganisms to improve soil characteristics (biofertilization) is one of the practices suggested by the EU to move to a sustainable agriculture thanks to the low environmental impact and the general effects on plant health (EU Sustainable Goals 2030: goals 3, 12, 15) that would ensure food security, re-establish soil biodiversity and ecosystems and recover arable lands (3).
This project aims at investigating the role of bacterial species present in commercial batches of compost (S.E.S.A. SpA) on tomato plants (*Solanum lycopersicum*) growth; the molecular pathways involved in plant-bacteria interaction; the bacteria colonization within plant organs. Firstly, bacterial strains have been isolated from the compost in order to characterize their biological diversity and their plant growth-promoting (PGP) traits. *Bacillus sonorensis*, *Bacillus subtilis* and *Kocuria rhizophila* are able to solubilize phosphorus (P) and to secrete siderophores, high-affinity iron-chelating compounds, and to synthetise the hormone auxin (IAA); *Glutamicibacter sp*. and *Microbacterium suwonense* produce siderophores and IAA; while *Bacillus licheniformis* was tested negative for these three traits. Then, the plant phenotypic response (Fig. 2) was evaluated by measuring various parameters of the roots, such as length and area, of tomato plants grown in soil enriched by the previously isolated bacterial strains and their mixture. Moreover, since plant-bacteria interactions induce great changes in plant morphology through different molecular pathways, a picture of the wide range of genes involved will be given by complementary strategies: the RT-qPCR will show the expression of specific genes involved in plant-microbe interactions; *in situ* hybridization technique will reveal the location of specific mRNAs in the tissues and the RNA sequencing analysis will present an overview of possible unknown genes regulating the process. Finally, tomato plants will be inoculated with bacterial strains transformed with fluorescent-tag proteins to visualise at confocal microscopy the stages of the symbiosis and the plant organs involved, as external and internal sides of the root, stem and leaves.
In conclusion, this study will contribute to decipher the complex network of the molecular signalling and regulatory genes beyond the interactions between plants and bacteria. Moreover, information about the best microbial consortia that promote plant growth will allow the improvement of the biological component of the compost for field application.

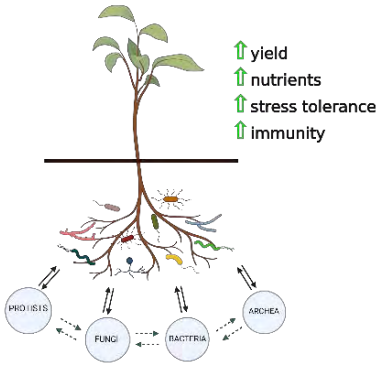


Fig. 1. Representation of the root.

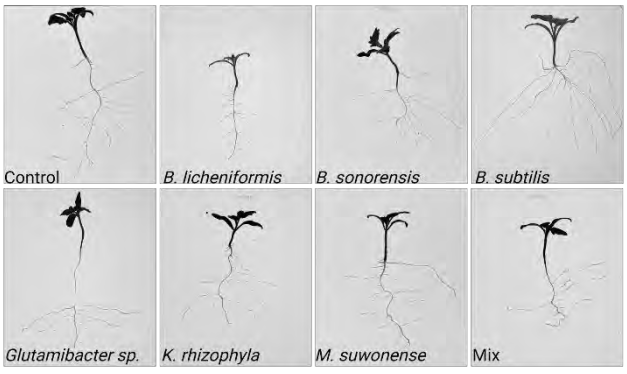


Fig. 2. 10 days-old tomato seedlings grown in soil inoculated with bacteria strains.

1) D. Bulgarelli, K. Schlaeppi S. Spaepen, E. Ver Loren van Themaat, P. Schulze-Lefert (2013) Annu Rev Plant Biol., 64, 807-38.
2) E. Baldan, S. Nigris, C. Romualdi, S. D'Alessandro, A. Clocchiatti, M. Zottini, et al. (2015) PLoS ONE, 10(10), e0140252.
3) United Nations (2015) https://wedocs.unep.org/20.500.11822/9814 (Accessed: 29 May 2023).

## 5.2 = Modulatory effect of non-thermal plasma-activated water on arbuscular mycorrhizal symbiosis

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Non-thermal atmospheric plasma, often named cold plasma, is characterized by relatively weak ionization with heavy particles (*e.g.* ions, molecules, radicals) at room temperature and electrons at a much higher mean energy. This condition of thermal non-equilibrium favours the presence of excited chemicals which, when interacting with water, result in a wide range of reactive oxygen and nitrogen species with various half-lives. This rich mixture of chemical species has been shown to stimulate biological responses when applied to living systems. In particular, the resulting plasma-activated water (PAW) has recently emerged as a safe, rather inexpensive, and eco-friendly alternative that may reduce the use of pesticides and fertilizers in agriculture, thanks to its antimicrobial/disinfection properties as well as to the reported effects on the improvement of seed germination and plant growth. Moreover, the ability of PAW to mildly induce plant defences, effectively boosting plant resistance against subsequent pathogen attacks (a pre-alert state termed "priming") is leading the research towards the fine-tuning of this novel "green" technology to maximise its beneficial effects in the context of a more sustainable agriculture (1).
Despite in the last few years there has been a surge of papers in this field, no information is currently available about how PAW treatment may impact plant-microbe symbiotic interactions in the rhizosphere.
In this work we have investigated the responses of the model legume *Lotus japonicus* to irrigation with PAW generated by a plasma torch, in terms of the establishment and development of arbuscular mycorrhizal (AM) symbiosis with the AM fungus *Rhizophagus irregularis*. Since PAW sensing by plants has been recently demonstrated to occur through calcium-mediated signalling (2, 3), in this study we have monitored early plant responses to different doses of PAW in *L. japonicus* roots expressing specifically targeted recombinant aequorin. AM fungal accommodation in the host plants was measured in terms of frequency, intensity of root colonization and percentage of arbuscules by the Trouvelot method. Chemical analyses of PAWs (in terms of $H_2O_2$, nitrates and nitrites) and plant samples (in terms of P, C, N, S) are currently being conducted, to determine the link between PAW composition and AM-mediated plant nutrient uptake.
The obtained results indicate that treatments with PAW play a modulatory role on plant AM symbiotic performance, in a manner dependent on the time interval of water exposure to cold plasma discharge and on the duration of plant irrigation with PAW. Establishing a sound scientific ground for cold plasma technology may provide key elements to develop tools and treatments aimed to increase crop plant yield in a sustainable way.

1) L. Holubová, S. Kyzek, I. Ďurovcová, J. Fabová, E. Horváthová, A. Ševcovicová, E. Gálová (2020) Int. J. Mol. Sci., 21, 9466.
2) E. Cortese, A.G. Settimi, S. Pettenuzzo, L. Cappellin, A. Galenda, A. Famengo, M. Dabalà, V. Antoni, L. Navazio (2021) Plants, 10, 2516.
3) E. Cortese, A. Galenda, A. Famengo, L. Cappellin, M. Roverso, A.G. Settimi, M. Dabalà, D. De Stefani, A. Fassina, G. Serianni, V. Antoni, L. Navazio (2022) Int. J. Mol. Sci., 23, 10752.

## 5.2 = *Lactuca sativa* genetic diversity shapes physiological and growth responses to a soil microbial synthetic community

Paolo Salvucci[1], Arianna Capparotto[1], Simone Sello[2], Cristina Sudiro[2], Adriano Altissimo[2], Marco Giovannetti[1,3]
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Phosphorus (P) is an essential mineral for plants and one of the most important plant growth-limiting nutrients. Despite its large abundance in the soil, less than 20% is present in the inorganic form, easily available for plants. To face this limitation, phosphate-based fertilizers have been intensively used in the agricultural field since the beginning of the first green revolution. However, their main P source derives from phosphate rock, which is a limited and non-renewable resource. In the last few years, microbial-based approaches have been proposed as a possible solution to increase sustainability in food production technologies. Among these, the most promising candidates are arbuscular mycorrhizal fungi, with their ability to dramatically extend the root surface involved in the phosphate absorption, and phosphate solubilizing bacteria. The aim of this project is to study the effect of a microbial inoculum, made by two arbuscular mycorrhizal fungi and a *Bacillus simplex* bacteria, on a panel of 128 fully sequenced varieties of *Lactuca sativa* in a controlled condition of P starvation, keeping in mind that plant genetic diversity plays an essential role in the responsiveness to microbial inoculi. Using a combination of physiological, metabolic and biomass parameters we were able to reconstruct the overall plant response to the inoculum. Considering all the lettuce genotype analysed, about 10% of these showed a statistically significant effect on plant growth and/or plant phosphate concentration, while the whole panel show a range of different responses highlighting the central role of genotype x environment interaction. The subset of *L. sativa* genotypes showing contrasting phenotypes will be further analysed for their photosynthetic pigments, free amino acids, total soluble sugar and starch content and a link to root microbial community will be investigated. Furthermore, all the phenotypic traits collected during the experiment have been used to perform genome wide association studies (GWAS). Preliminary results demonstrated that SNPs located in chromosome four of *L. sativa* genotype are associated with differences in soluble phosphate accumulation upon soil inoculum. In addition to that, two genetic loci in chromosome two and five, respectively, could be involved in the diverse effect of the inoculum on shoot biomass. To conclude, the use of microbial-based consortium represents a step forward toward the development of more sustainable techniques in agriculture but a deeper understanding of the interaction between soil microbes and plant genetic diversity is needed. With this study, we aim to add a new piece of knowledge on the genetic and physiological mechanisms underlying the establishment and the effects of beneficial interaction between plants and soil microorganisms.

## 5.3 = Large-scale bioprospection of the Italian flora for specialized metabolism mapping and biodiversity exploitation

Flavia Guzzo[1], Linda Avesani[1], Mauro Commisso[1], Leonardo Bisson[1], Valentina Dusi[1], Fabio Pietrolucci[1], Fabio Pinzauti[1], Carolina Ramos[1], Gianluca Zorzi[1], Sebastiano Nigris[2], Barbara Baldan[2], Sebastiano Andreatta[3], Anna Lisa Piccinelli[4], Rita Celano[4], Maria Carmela Caria[5], Alfredo Maccioni[6], Emmanuele Farris[5], Simonetta Bagella[5], Stefano Negri[1]

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The biodiversity is of enormous value not only for the health and resilience of ecosystems, but also as a source of valuable biomolecules potentially able to improve human health. In the frame of the National Biodiversity Future Center, dedicated to monitoring, conservation, restoration, and exploitation of biodiversity, a wide bioprospection plan has been setup, aimed at mapping the specialized metabolisms of the Italian flora. This plan includes selection and sampling of 600 species representing all the families of the Italian vascular and non-vascular flora, their characterization through state-of-the-art High Resolution Liquid Chromatography-Mass Spectrometry, and the selection of the species with the more interesting chromatographic profiles for a downstream bioactivity screening program, mainly focused on non-communicable diseases.

The comparison of all the species through untargeted metabolomics will also be performed and will be used to map the specialized metabolisms and the related genes through all the families of actual wild Italian flora, including indigenous, archaeophytes and naturalized neophyte species. To enrich the more relevant evolutive crossroad, for instance within the group of basal Angiosperms and within the Gymnosperm orders, also a small group of non-Italian species have been included.

This extensive mapping has two widely interconnected purposes: to gain greater knowledge on the specialized metabolites of land plants and their evolution, and to build a library (physical and *in silico*) of molecules that could be exploited to produce drugs, nutraceuticals, cosmetic and products for a more sustainable agriculture.

## 5.3 = The “Ortano artichoke” and its valorization as a source of bioactive compounds: studies on the effects of methanolic extracts in *in vitro*, 2D and 3D cell culture models

Valentina Laghezza Masci[1], William Tomassi[1], Irene Mezzani[1], Paolo Mancuso[1], Vittorio Vinciguerra[1], Antonio Tiezzi[1], Elisa Ovidi[1]
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Technological innovations in agriculture are moving towards a circular system, based on production processes that allow for better sustainability and on biotechnological methods for the recovery and valorization of waste and by-products. Among the agricultural crops, the artichoke produces a lot of waste, such as leaves, stems, and roots, and up to 80 % of the total biomass produced is discarded (1).
*Cynara cardunculus* L. var *scolymus* L. is a plant belonging to the Asteraceae family, native to the Mediterranean region and cultivated worldwide for its nutritional benefits and medicinal properties. The economic use of the crop mainly includes consumption of the immature edible flower heads, which are highly valued for their organoleptic and nutraceutical properties. The health properties of artichoke extracts have been studied, even from non-edible portions of the plant and numerous commercial products of pharmaceutical and medical interest are used (2).
In order to reuse waste and by-products as source of molecules with potential biological activities, the extracts from four organs (leaves, stems, principal and secondary flower heads) of a local variety of *C. cardunculus* from Latium region (Italy) were investigated. This variety, called “Ortano artichoke”, was characterized thanks to genetic analysis that confirmed the belonging to the “Romanesco” varietal typology and revealed a high level of genetic variability within this landrace (3).
Two representative genotypes of the Ortano variety (named, respectively, Orte 1 and Orte 2) were characterized in terms of polyphenolic content by HPLC-DAD analysis and the biological activities studied.
To define the antioxidant activity of the extracts, FRAP, ABTS and DPPH tests were performed, as well as the determination of ROS production and GSH level in a live cell assay of human neuroblastoma SH-SY5Y cells treated with stems and secondary flower heads extracts, which were found to be the most active.
Furthermore, cytotoxicity and antiproliferative activity on human adenocarcinoma cell lines, MCF-7 and MDA, were studied by MTT assay in 2D models. Knowledge about the biological activity of plant extracts is usually derived from 2D studies, however the development of 3D models is on the rise.
For this purpose, here we compared 2D cell models with 3D models by means of Live-Dead assay, laser scanning confocal microscopy (CLSM), scanning and transmission electron microscopy (SEM and TEM). The studies revealed biological activity on the cell cycle by leaf extracts, flow cytometry and western blot analyses confirmed the induction of cell cycle arrest.
In conclusion, the potential use of wastes and by-products as a source of bioactive compounds was highlighted in this research, and further study will be needed to optimize the extraction method for large quantities of agricultural waste from this crop.

1) V. Lattanzio, P.A. Kroon, V. Linsalata, A. Cardinali (2009) J Funct Foods., 1(2), 131-144. DOI.org/10.1016/ j.jff.2009.01.002.
2) A.R.S. Mateus, A. Pena, R. Sendón, C. Almeida, G.A. Nieto, K. Khwaldia, A.S. Silva (2023) Trends Food Sci Technol., 131, 220-243. DOI.org/10.1016/j.tifs.2022.12.004.
3) E. Alicandri, A.R. Paolacci, G. Catarcione, A. Del Lungo, V. Iacoponi, F. Pati, G. Scarascia Mugnozza, M. Ciaffi (2023) Plants., 12(9), 1844. DOI: 10.3390/plants12091844.

## 5.3 = Anti-inflammatory and neuroprotective potential of *Cannabis sativa* essential oils in a microglia cell model

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*Cannabis sativa* L. essential oil (EO) is a complex mixture of volatile compounds obtained from the plant inflorescences, an added-value by-product usually discarded from the hemp supply chains. The chemical composition and hydrodistillation yield of the hemp EO are influenced by both the hemp genotype and the year of cultivation, as well as by the plant phenological stage (1,2). Despite its variability, terpenes and cannabinoids represent the main chemical classes of the hemp EO; among the former, sesquiterpenes tend to be the most variable components, both in their hydrocarbon and oxygenated forms (1,2). Since biological activities are strictly related to the content of secondary metabolites, the aim of the present work was the evaluation of the ability of the EOs obtained from two industrial genotypes of *C. sativa* characterized by different chemical compositions to counteract the microglia-mediated neuroinflammation in a human microglia cell model (HMC3).

The EOs were obtained by hydrodistillation in a Clevenger apparatus from the air-dried apical parts of the selection S435 and the cv. Eletta Campana, cultivated in an open field in Bologna (Italy) and harvested at seed maturity. The EOs were analysed by Gas Chromatography coupled with Mass Spectrometry, and then evaluated for their cytotoxicity, ROS production, and influence on pro- and anti-inflammatory cytokines in a microglia cell model.

The EOs were characterized by a similar relative abundance of cannabinoids, whose major component was represented by cannabidiol (CBD). Conversely, sesquiterpenes, both in their hydrocarbons and oxygenated forms, showed great differences in terms of their relative amounts: the former class was more abundant in Eletta Campana, while the latter in S435. According to literature data and to our previous studies, β-caryophyllene, α-humulene, and caryophyllene oxide represented the most important compounds detected among these classes. Concerning the cytotoxicity assay, Eletta Campana and S435 EO decreased the viability of HMC3 cells at concentrations of 50 or 100 μg/ml respectively, while lower concentrations did not show a significative difference from the control. Since one of the mechanisms responsible for cell death is the accumulation of ROS, the ability of the EOs to influence their production was also assessed, although neither reduction nor increase in the ROS levels were shown. Finally, the investigation of the influence of the EOs on the gene expression of the pro-inflammatory cytokines IL-6 and IL-8, the anti-inflammatory cytokines IL-10, and the inflammatory transcription factor NFkB was performed by real-time RT-PCR. Eletta Campana, richer in sesquiterpene hydrocarbons, showed a greater anti-inflammatory activity, as it simultaneously decreased the expression of IL-6 and IL-8 and increased that of IL-10. Conversely, S435, characterized by a prevalence of oxygenated sesquiterpenes, did not show the same outcomes.

1) Y. Pieracci, R. Ascrizzi, V. Terreni, L. Pistelli, G. Flamini, L. Bassolino et al. (2021) Molecules, 26, 4080.
2) Y. Pieracci, F. Fulvio, V. Isca, L. Pistelli, L. Bassolino, M. Montanari et al. (2023) Ind. Crops Prod., 197, 116605.

## 5.3 = Unlocking the biorefinery potential: enhancing process design and optimization for bioactive extraction from pomegranate marc using super- and subcritical fluids

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The industrial technology used for extracting pomegranate juice by hydraulic pressing generates significant amounts of pomegranate marc (PM). This by-product consists of rinds and kernels that are exceptionally rich in lipo- and hydro-philic compounds. Notably, dehydrated PM powder contains up to 6.1% oil, primarily from the kernels, which includes polyunsaturated fatty acids such as conjugated α-linolenic acids (CLnA). Additionally, it contains substantial amounts of phenolics and dietary fibers from both kernels and rinds (1). These valuable compounds can be efficiently recovered through biorefining strategies to obtain high-quality ingredients suitable for pharmaceutical, cosmeceutical, and nutraceutical applications. This utilization of the agri-food by-product contributes to the sustainability of pomegranate production and processing, aligning with the principles of the circular economy.
Supercritical carbon dioxide extraction (SFE) and subcritical water extraction (SWE) are environmentally friendly technologies that complement with each other in the effective extraction of valuable lipo- and hydro-soluble natural molecules. These technologies are gaining popularity in industrial production of solvent-free plant phytocomplexes. However, the successful industrial application of SFE and SWE requires careful optimization of operational parameters to ensure economic and efficient processes (2).
To address this, a Response Surface Methodology approach based on the Box-Behnken design was employed to model and maximize the sequential extraction of PM oil (oil yield %) and phenols (total phenols %) using SFE and SWE, respectively. The main operational parameters, including temperature, pressure, solvent flow, and extraction time for SFE, and temperature, solvent/solid ratio, (with constant pressure at 40 MPa, based on literature data) for SWE, were optimized during the study.
The SFE experimental data were fitted to a second-order polynomial equation by multiple regression analysis and analysed using appropriate statistical methods. The regression coefficients of the intercept, linear, quadratic, and interaction terms of the quadratic model, determined through the least square method, revealed that temperature (B) and its square ($B^2$) were the most significant model terms ($p < 0.05$). $CO_2$ flow (C and $C^2$), time (D and $D^2$) and pressure (A) followed insignificance. As for $A^2$ and all factor interactions, they were deemed insignificant and excluded from the equation to improve the model. Instead, the SWE data were best described by a first order linear equation with temperature (A) as the most significant parameter.
The 3-D response surface plots derived from the mathematical models of SFE and SWE were applied to determine the optimal extraction parameters: 43 MPa pressure, 76°C temperature, 6.4 L/min expanded $CO_2$ flux and 124 min time for SFE, and 149°C temperature, 40 L/kg solvent/solid ratio, 73 min time for SWE. Under these conditions, the experimental yield of oil was 3.9 g·100 g dry weight (dw) $^{-1}$, approximately 64% of the total oil content of the dried PM matrix (as estimated directly by extensive soxhlet extraction with ethyl acetate), and in good agreement with the model predicted value (68 ± 24%). The experimental yield for total phenols was 4.4 g 100 g $dw^{-1}$, corresponding to 54% of the total phenols of the dried PM (measured by extensive maceration extraction with 80% ethanol), in good agreement with the prediction (54 ± 9%).
The PM oil extracted under the optimized operating parameters mainly contained punicic acid and other CLnA, which accounted for over 70% of the total identified fatty acids. These compounds are well-known for their anti-inflammatory, immunomodulatory, anti-cancer, and anti-oestrogen effects. The oil also contained phytosterols (9.50 ± 1.02 mg/g), β-tocopherol (3.77 ± 0.92 mg/g), small amounts of phenol compounds (0.42 ± 0.13 mg/g), and traces of triterpenes, biomolecules with potential roles in maintaining human health and preventing degenerative diseases. The phenolic profile of the SWE extract is currently being characterized.
Overall, our results prove that the newly developed biorefinery process is suitable for the valorizing the PM by-product. Under the optimized operating conditions, it efficiently extracts value-added fractions with potential antioxidant and bioactive activities, while also producing an exhausted residue rich in insoluble fibers (primarily cellulose) that can be utilized by the food/feed industry or as a soil conditioner.

1) A. Montefusco, M. Durante, D. Migoni, M. De Caroli, R. Ilahy, Z. Pék, L. Helyes, F.P. Fanizzi, G. Mita, G. Piro, M.S. Lenucci (2021) Plants (Basel), 10(11), 2521.
2) M. Durante, A. Ferramosca, L. Treppiccione, M. Di Giacomo, V. Zara, A. Montefusco, G. Piro, G. Mita, P. Bergamo, M.S. Lenucci (2020) Food Chem., 332, 127405.

## 5.4 = Deepening plant microbial fuel cells' roots: plant nomenclature, and how life forms and root architecture affect bioelectrical performances

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Plant microbial fuel cells (PMFCs) represent a promising biotechnology in the context of energy harvesting. PMFCs are a derived technology of microbial fuel cells (MFC), which uses plant rhizodeposition as nourishment for the electrochemically active bacteria (EAB) growing on the surface of the anode and enabling the generation of bio-electricity [1]. Choosing the proper species is crucial to maximize the bioelectrical performance. Some of the criteria for the selection of plants may ideally include hardiness and extensiveness of the root system. PMFCs were initially restricted to aquatic plants and indoor ornamental plants, mainly herbaceous. They have been slowly extended to trees in the last 5 years. We will focus on the herbaceous species, except for some well-tested Epiphytes, Nanophanerophytes and Chamephytes, as tree experimentation is in an early stage. The most of recent reviews about PMFCs analyze engineering and microbiological aspects, while the botanical aspect has often been neglected. One of the most underrated aspects concerns the correct nomenclature of the species used in PMFCs, making difficult to trace back to the species really tested. Thus, this review analyzes some botanical aspects of species used in PMFCs with the aim to verify if their electrical performances vary according their different life forms (which imply different biomasses providing nutrients for EAB) and root systems (around which microbial communities develop).
We investigated, for each species used so far: I) Nomenclature; II) Raunkiær life form; III) Root architecture. We used the most relevant reviews (found by using Clarivate search tool) to find the original articles in which PMFC experimentation where described and we selected papers containing pertinent information on plant species. We found out a total of 124 entities used for PMFCs. We excluded 8 plants (including 2 Pteridophytes) defined only at *genus* level, 11 Bryophytes, 21 Phanerophytes and lignified shrubs. Due to nomenclature doubts, we also excluded other 5 entities. We obtained a list of 79 species and 58 documents useful for further processing. Every plant name, and relative Raunkiær life form, was verified in POWO [2] and checked in the Flora of a native Country. We divided root architecture types into three groups representative of the abundance and structural complexity of the hypogeal apparatus: 1) Taproot, Tuberous and Bulbous; 2) Rhizomatous, Stoloniferous and Adventitous; 3) Fibrous, Fasciculated, Branched. We based the groups' assignment on "root type architecture" trait verified by a detailed study on TRY (Plant Trait Database) [3], when available for the species, checked in the Flora of a native Country or expert based. Concerning the electrical performance data collection, species were excluded from the study in the following cases: a) there was no correspondence between electrical performance and specific plant in the experimentation; b) electrical performance was not calculated or published; c) only voltage or current value was provided; d) average power and peak power values were too similar; e) power density values were $< 1$ $\mu W/m^2$ or $> 950$ $mW/m^2$. When more than one measurement was available for a species, the one with the highest value was considered. We obtained a final list of 46 species and 42 documents.
We found significant differences between the Epiphyte/Chamaephyte/Nanophanerophyte group and Geophytes, with Therophytes and Hemicryptophytes showing the highest median values. We also found significant differences among the root architecture groups, with the group 1 showing the highest median value. These results seem to highlight that electrical performances are affected by life forms and root systems. Anyway, these results may have suffered by some limitations due to the lack of a common benchmark for electrical measures, implying a necessary approximation of power density values.

1) F.T. Kabutey, Q. Zhao, L. Wei, J. Ding, P. Antwi, F.K. Quashie, W. Wang (2019) Renewable and Sustainable Energy Reviews, 110, 402-414.
2) POWO (2023) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://www.plantsoftheworldonline.org/ Retrieved 25 January 2023.
3) J. Kattge, G. Boenisch, S. Diaz et al. (2020) Glob Change Biol., 26, 119-188. https://doi.org/10.1111/gcb.14904

## 5.4 = The influence of light in seeds quality and secondary metabolites regulation during fruit and seed development

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It is known that light plays a dual role for the plant: a source of energy through photosynthesis and a mechanism by which the plant perceives the surroundings environment.
The specific response triggered by different light qualities and intensities, alter the primary as well the secondary metabolism of plant. Therefore, they can be applied to induce the synthesis of selected compounds, in order to improve the nutritional quality of horticultural crops (1, 2).
Here, we report how the light quality affects seeds and fruit quality in the model plant *Nicotiana tabacum* L. Specifically, fruits development and ripening under the blue (390 nm), or the red (650 nm) regions of the electromagnetic spectrum were tested. Positive and negative control were represented by capsules of plants grown in presence of White light, and etiolated capsules developed under dark conditions, respectively (3).
Seeds and fruits obtained from the different light conditions were evaluated by morphometric and colorimetric analysis. Furthermore, an experimental protocol was optimized for the simultaneous evaluation of different phytohormone classes using a small amount of seeds. Specifically, ACN extracts from seeds were purified and then analyzed by LC-MS/MS, allowing us to evaluate the alteration in phytohormones content. The analyses were performed with up to 2 mg of dry tobacco seeds, testing simultaneously 80 compounds, among which 30 were detected in the seeds under different experimental conditions.
Considering the differences in seeds weight, their lipid profiles were then analysed to highlight light-induced alterations in nutrient storage. These results were then compared with the hormonomics evaluation, which allowed a comprehensive view not only of the active hormones, but also of their precursors, of their conjugated forms as well as of their related substances, giving an overall landscape of the metabolic alteration.
Therefore, this work aims at exploiting the mechanisms of response to light and stress stimuli developed by plants during evolution. The mechanisms involved are analysed taking into account the role that hormones and their metabolites play in fruit and seed production, as they regulate not only their maturation, but also their nutrient content and storage. In view of the promising preliminary data obtained, the ultimate goal is to use the same approach to other species of the Solanaceae family (e.g. genera *Solanum*, *Capsicum*) as an applicable *proof of concept* to other species of economic interest.

1) H.R. Soufi, H.R. Roosta, P. Stępień, K. Malekzadeh, M. Hamidpour (2013) Sci. Rep., 13, 1-8600.
2) R. Paradiso, S. Proietti, S. (2022) Journal of Plant Growth Regulation, 41(2), 742-780.
3) E. Cocco, D. Farci, P. Haniewicz, W.P. Schröder, A. Maxia, D. Piano (2022) Seeds, 1(3), 152-163.

## 5.4 = Isolation of synthetic binders for the identification and evaluation of forest plant stress biomarkers

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Forest environments present highly fluctuating conditions that impact tree growth and development. Plants are exposed to multiple stressful conditions due to both abiotic and biotic factors, such as high light intensity, air pollutants, high salt concentration, drought, pathogens. Understanding the molecular mechanisms triggered by stress conditions and monitoring the fluctuations of such biomarkers with affordable methods would be instrumental for assessing stress levels over the time and possibly prevent massive decline of forest trees, commonly associated with multiple sustained stresses (1). The evaluation of plant physiological conditions can be performed by applying different methods and our contribution is the introduction of new molecular approaches for identifying useful biomarkers and suitable reagents for their reliable evaluation. In our research, we first optimized experimental procedures on *Pisum sativum* to move then to *Poplar* plants. Two different panning strategies were used to recover binders specific for stress plant biomarkers: (i) blind biopanning against whole cells of *Pisum* protoplasts and (ii) biopanning against recombinant soluble antigens such as ascorbate peroxidase. In the first case, we isolated adhirons, synthetic ligands of less than 100 amino acids designed starting from consensus sequence of plant phytocystatin (2). In the second case, nanobodies were recovered. Once ligands (adhirons or nanobodies) were selected, they were expressed recombinantly and evaluated for their functional and biophysical characteristics. Binding specificity and affinity of adhirons/nanobodies for their antigens were determined by ELISA immunoassay, gel filtration and by Surface Plasmon Resonance. Moreover, the characterization of complexes between adhirons and protoplast antigens is under investigation by means of microscopy techniques. The expertise will be then applied to tree cells with the aim to develop a molecular approach that should speed up the research of the biological mechanisms relevant for forest plants.

1) G.M. Walters, B.C. McCarthy (1997) Ohio J. Sci.97, 5-9.
2) C. Tiede, A.A.S. Tang, S.E. Deacon, U. Mandal, J.E. Nettleship, R.L. Owen, S.E. George, D.J. Harrison, R.J. Owens, D.C. Tomlinson, M.J. McPherson (2014) Protein Eng. Des. Sel., 27, 145-155.

## 5.4 = Overcoming the micronutrients depletion of aquaponics' cultivation: *Arthrospira platensis* as a foliar fertilizer for *Lactuca sativa* plants

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Aquaponics represents the fusion of hydroponic cultivation techniques with aquaculture, in which water recirculates within the system. One of the major problems of aquaponic cultures is represented by the depletion of nutrients over time, iron above all. This work made it possible to test a spirulina-based foliar biofertilizer, able to compensate for the lack of micronutrients. The experimental aquaponics system used tilapias (*Oreochromis niloticus* L.) as fish, bacteria capable of oxidizing waste ammonia into compounds usable by plants, such as nitrites and, above all, nitrates, and *Lactuca sativa* L. cv. Foglia di Quercia Verde.
An industrial waste was used for this purpose, in detail the supernatant coming from a spirulina cell lysate. Macro and micronutrients (N, P, K, Fe, Cu, Mn, Mg, S, Mo, Cl, Zn, Ca) were analyzed in the supernatant fraction and in the water present in the cultivation system. The supernatant was considered as starting stock solution to prepare the dilutions (100%, 75%, 50% and 25%) used later in the experiments as foliar fertilizer. Sterile distilled water was used as a control group. Experiments were carried out from November 2022 to January 2023 at the aquaponics greenhouse of the Botanical Gardens of the University of Rome "Tor Vergata" (Rome, Italy).
*L. sativa* plants were harvested after 65 days from sowing. Fresh and dry weightings were performed both on leaves and roots. Plants were photographed using a Canon EOS 550D and acquired pictures were processed for determining the length of leaves and roots. The analysis carried out on the cultivated plants involved the soluble solids content (SSC), the photosynthetic pigments (chlorophyll a, b, and carotenoids), phenols and flavonoids, each following protocols reported in the literature (1, 2, 3).
The evidence found in this study showed that the 75% dilution of the biofertilizer used had the most significant effect on the growth of *L. sativa*. In fact, data on shoot length showed that 75% treatment was more effective than the other, similarly, fresh shoot weight had same trend. Even for total weight, the 75% group was statistically significant higher compared to other dilutions. The morpho-biometrical data obtained from this experiment showed that the spray application of spirulina-based biofertilizer promoted more shoot growth rather than root growth. This has a strong significance at both a productive and economic level, as leaves are the portion that is consumed.
Also, for both qualitative and quantitative aspects involved soluble solids, chlorophylls, carotenoids, and phenols contents both 75% and 100% treatment showed the best results.
The content of macro and micronutrients in the supernatant portion of the spirulina lysate used in this study was therefore found to be suitable for promoting the growth of lettuce plants, also acting at level of photosynthetic pigments by increasing their synthesis. The higher phenolic content detected in the treated plants provided further evidence of the efficacy of spirulina as a biofertilizer to produce quality *L. sativa* grown in aquaponics. The data relating to the total flavonoid content differed from the trend observed for the other parameters; in fact, the situation in this case was the opposite.
The data obtained from this study made it possible to outline the spectrum of action of a biofertilizer based on a spirulina-industrial waste's extract on *L. sativa* 'Foglia di Quercia Verde' plants grown in an aquaponic system. Thanks to the comparisons with the control group and those analyzed between the various administrations of this spray fertilizer, it was possible to identify the best treatment for the tested plants. This study has provided a sustainable alternative in favor of a circular economy of resources, offering new ideas for the application of biofertilizers made with waste products, making up for the lack of nutrients that inevitably occurs in aquaponic cultivation systems.

1) R. Braglia, P. Costa, G. Di Marco, A. D'Agostino, E.L. Redi, F. Scuderi, A. Gismondi, A. Canini (2022) J. Sci. Food Agric., 102, 844–850.
2) H.K. Lichtenthaler (1987) Methods Enzymol, 148, 350–382.
3) C.C. Chang, M.H. Yang, H.M. Wen, J.C. Chern (2002) J. Food Drug Anal., 10, 178–182.

## 5.4 = Isolation and characterization of novel halotolerant rhizobacteria with plant growth promoting features and their counteracting action against salt stress on *Zea mays*

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The salinization of soils is the most relevant challenge for the agriculture in 21st century. The soil salinity due to natural phenomena or human activities, consist in a progressive accumulation of salt into the soil negatively influencing the plant growth and its productivity. Consequently, it is important to develop new biotechnologies to counteract this problem. An environmentally friendly approach to ameliorate the cultivation of crops in contaminated salt soil, could be the use of Plant Growth Promoting Rhizobacteria (PGPR) that can improve plant growth by colonizing their roots and playing a vital role in helping plants to establish and grow in adverse conditions. Our research aimed to isolate, characterize, and test in *in vivo* these microorganisms in soil affected by NaCl. Initially, among 16 different rhizobacteria were isolated from rhizosphere of two different plant species (maize and quinoa). The16S rDNA sequences analysis showed that they are all distinct strains and two of them would belong to two new bacterial species. Afterward, we characterized biochemically these bacteria based on the main relevant plant growth promoting features (*e.g.*, indol-3-acetic acid, ammonia, siderophores, phosphatases and ACC-deaminase production). Moreover, we observed that some of the isolated strains exhibited some of these features also in a salt-dependent manner. Finally, we tested two isolated strains that exhibited many PGP features: *Bacillus stratosphericus* and *Staphylococcus succinus*, in *in vivo* experiment inoculating them on maize seedlings. The bacterial inoculation was performed using the seed-coating method, and the related effects were evaluated by morphometric analysis, quantification of ion amount (sodium, potassium), produced biomass, both for epigeal (shoot) and hypogeal (root) organs, and by measuring salt-induced oxidative damage. Finally, we demonstrated that the co-treatment with both B. *stratosphericus* and *S. succinus* increased biomass and sodium tolerance and even we observed a reduction of oxidative stress over the Control group. Moreover, bacterial treatment partially restored the root architecture system damaged and altered by the presence of salt in the soil when compared to the Control group. Therefore, the isolation and characterization of new PGPR could be consider potentially useful in agriculture to improve the tolerance and productivity of crops in those soils affected by high content of salt.

## 5.4 = Alternative fertilization through the use of functionalized biochar in tomato plants

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Current scientific research focuses on the study and production of specific fertilizers to improve soil fertility and product quality while maximizing nutrient assimilation and minimizing environmental impact. However, specific chemical fertilizers are often required to restore fertility to depleted, exploited, or arid soils. The use of chemical substances to provide micro and macronutrients necessary for proper plant development and productivity maximization is currently the most widespread agricultural practice. Plant growth and development depend primarily on the bioavailability of nutrients, and the choice of fertilizer composition (micro and macronutrients) depends on the specific needs of each plant. However, several studies conducted in recent years have highlighted the environmental issues associated with the use of traditional fertilizers. Globally, 138 million tons of chemical fertilizers are used each year, resulting in increased water pollution, particularly regarding nitrogen (N) and phosphorus (P) elements. When a commercial fertilizer is applied, it exhibits low nutrient bioavailability, leading to the accumulation of unabsorbed nutrients in the soil and water bodies, which can cause water eutrophication. To address these issues, research is developing innovative methodologies such as functionalized biochar. Biochar is a porous carbon-rich material obtained from the pyrolysis of biomass, such as food processing waste, animal manure, or crop residues. Research on the use of biochar as a fertilizer has increased in the past two decades. Its high carbon (C) content makes it an excellent soil fertilizer as it can restore soil microbiome, unlike commercial fertilizers that deplete it. In addition to the observed benefits on plant growth, biochar also has the ability to retain nutrients, reducing leaching. The biochar tested in our experiments was derived from the waste of the licorice industry. Specifically, this waste was pulverized before undergoing pyrolysis at 500°C. Once the biochar was obtained, its structure was observed using scanning electron microscopy (SEM) (Fig. 1). The biochar was then functionalized through an impregnation process with the main macronutrients required by plants for growth, namely nitrogen, phosphorus, and potassium, with the following two ratios: BCA (2:4:8) and BCB (4:1:3). The experimental design included not only different types of biochar, including untreated biochar, but also different soil-to-amendment (biochar) ratios to observe any differences due to the amount of biochar used relative to the total amount of soil present in the pot. The plant used in the experiments was *Solanum Lycopersicum*. The experimental design included 4 replicates for each treatment, and the plants were observed for a growth period of 28 days from sowing. From the results obtained, it was possible to demonstrate that after 28 days, all the plants grown in the presence of biochar were significantly taller than the controls grown in soil alone, with up to 90% more length compared to the control (BC0-15%). Additionally, the amount of chlorophyll and carotenoids in the leaves was also evaluated using a spectrophotometer. From the results, it can be concluded that the total chlorophyll content in plants grown in the presence of BCO and BCB at all tested ratios (5-15-25%) showed a significantly higher amount of chlorophyll compared to the control. Furthermore, the possible pH change in the presence of untreated and functionalized biochar was also evaluated compared to the control. After 28 days, the pH of the soils amended with biochar was more acidic compared to the control, which had a more neutral pH. This aspect ensures a better growth environment for the plant and availability of elements.

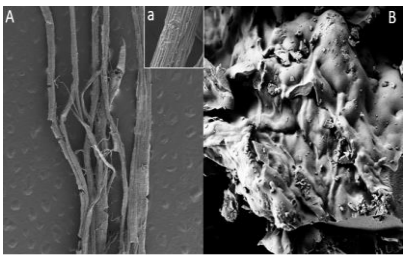


Fig. 1 (A-a,B). Fig. A. shows a SEM image of the licorice fragments used, and image a shows a detail of the fragments. Fig. B. shows a SEM image of the biochar obtained from the pyrolysis process.

## 5.5 = Ecological analysis of the biodeteriogen vascular flora: a study case on the ancient castles and towers of Campania

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Biodeterioration is defined as the alteration of a given material by a variety of living organisms such as bacteria, fungi, lichens, mosses, and higher plants that cause physical and chemical reactions damaging historic buildings and stone monuments. This phenomenon is a widespread problem affecting two-thirds of the world's cultural heritage (1), reason why its preservation has become a concern of science. The aim of the present study is to evaluate the colonizing vegetation, with a focus on vascular plants, growing on ancient castles (N. 70) and towers (N. 30) located in Campania in correlation with different building materials, exposition, elevation, distance from the sea, level of maintenance, and surrounding vegetation. Samples representing the various floristic types in terms of plant cover and floristic diversity, measuring one square meter each, are analysed for each study site. These samplings are strategically placed at the different exposures of each substrate locally present. Within each sampling units the following data are provided: position (UTM coordinates), substrate, position (vertical or horizontal) and exposure. Plant cover is recorded by the Braun-Blanquet abundance–dominance scale (2). Preliminary data concerning root development under conditions simulating the growth in cracks of some biodeteriogenic plants have been obtained. To date 20 sites have been studied, where the most common species are *Dittrichia viscosa, Micromeria graeca, Cymbalaria muralis, Centranthus ruber*, and *Parietaria judaica*. The main differences include the presence of *Bituminaria bituminosa, Phagnalon rupestre, Olea europea* and *Pistacia lentiscus* in sites closer to the sea, and presence of *Geranium rotundifolium, Reseda alba, Fraxinus ornus* and *Ostrya carpinifolia* in sites of more internal areas. At the end of this work an overview of the vegetation on castles and towers in Campania will be obtained, observing the floristic distribution based on the variables analyzed during the study. Overall, this work is a valuable resource and provides a solid basis for further study and implementation of initiatives to enhance Campania’s cultural heritage and improve its preservation and conservation.

1) R. Motti, G. Bonanomi (2018) International Biodeterioration & Biodegradation, 133, 26-33.
2) J. Braun-Blanquet (1932) Plant sociology. McGraw-Hill Book Company, New York and London.

## 5.5 = New investigation on the historical botanical collections of the Royal Villa of Monza

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The aim of this work, funded by the project "Scienza Viva. Botanical Gardens and Gardens of Delight. Antique knowledge and good practices for the diffusion of Scientific Culture" (Orti Botanici e Giardini di Delizia. Antichi saperi e buone pratiche per la diffusione della cultura scientifica) is to perform new investigations on the historical botanical collections of the Royal Gardens and the Park of the Royal Villa of Monza (Monza, Lombardy), one of the former residences of the Italian Savoy Royal family. The Royal Gardens and the nearby Monza Park are important cultural and natural heritages and together they constitute the largest walled park in Europe reaching 720 hectares, almost three times the Park of Versailles. The story of this wide green area began with the Archduke Ferdinand of Habsburg, who chose to build his own countryside mansion in Monza. The building of the Royal Villa of Monza and the gardens took place between 1778 and 1783: Giuseppe Piermarini was the architect engaged for this project. In particular, he designed the Royal Gardens with the idea of giving a unified perception with the surrounding landscape, creating the first English landscape garden in Italy. In 1796 the Lombardy territory was conquered by the French, and in 1805 Napoleon's adoptive son, Eugène de Beauharnais, gave the task of projecting the Park to Luigi Canonica (the National Architect of the French Court) and to the gardener Luigi Villoresi with two aims: to establish a model farm and to realize a hunting reserve for the Viceroy. In 1859 the Royal Villa became the Savoy's residence, but after the murder of the Italian King Umberto I in 1900 in Monza, his son Vittorio Emanuele III decided to abandon this residence. From that moment the Villa and the Park started to decline (1). Since 2009 the Royal Gardens and the Monza Park have been managed by the *Consorzio Villa Reale e Parco di Monza*, which is composed of the owner institutions of Monza Park and Royal Villa: the Ministry of Culture, the Lombardy Region, the Municipality of Monza and the Municipality of Milan who deal with their valorization.
The plant diversity of this green area has been relevant over the years, thanks to the gardeners who have introduced many species. The first catalogue was realized by Luigi Villoresi in 1813 (*Catalogus plantarum existentium in hortis Regiae Villae prope Modoetiam*); it includes about 3,700 specimens and 34 genera of tree species. In 1826 Giovanbattista Rossi realized the *Catalogus plantarum horti regii modoetiensis* accounting 38 tree species genera, followed by the catalogue by Giuseppe Manetti in 1846 (*Catalogus plantarum caesarei regii horti prope modiciam*) that includes around 10.000 specimens (2). According to historical documents, the Archduke of Austria Joseph Ranieri, the Viceroy of the Lombard-Venetian Kingdom (1818-1848), was a great lover of natural sciences and promoted and stimulated botanical activities and agronomic experiments. In particular, during this period the *Herbarium Rainerianum* was realized. It is the result of the intense exchange of exsiccata between the most important botanists of that time, and together with Gaetano Grigolato's *Flora Policinensis* and Agostino and Carlo Perini's *Flora Tridentina* became part of the herbarium of the Royal Villa (3). The Archduke Ranieri also established a collection of wooden "booklets" with the aim of documenting the shrub and tree flora of the Botanical Garden of the Royal Villa. This collection was found in 1935 and it converged in the *Siloteca Cormio* (1935-1955), an important collection of tree and shrub samples carefully prepared with rationality and an aesthetic sense. Today all this historic and scientific material is stored at the Natural History Museum of Milan (3).
These historical catalogues are records of the progress of botanical science and are useful instruments for reconstructing the relationships that were established between enlightened people. Also, the catalogues are a very interesting source for the history of the introduction of exotic plants, which were cultivated and reproduced for their ornamental features.

1) C. M. Zerbi, A. Cazzani, L. S. Pelissetti, L. Scazzosi (2016) "Valorizzare il patrimonio dei giardini storici lombardi attraverso lo sviluppo di una rete sostenibile di competenze - Il Piano di Gestione Programmata come strumento operativo", ReGiS, 109.
2) F. Pizzoni (2014) Il Parco, la Villa, Quaderno 7, 58-66.
3) E. Banfi, R. Cassanelli, S. Sicoli (2000) "Herbarium Rainerianum", Silvana Editoriale.

## 5.5 = The historic garden Villa De Capoa: between biodiversity and sociability

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The University of Molise is working with Campobasso municipality to restore and improve a historic garden as part of the PNRR M1C3 project. The project name is: "*The historic garden of Villa De Capoa: between biodiversity and sociability"* and it adheres to the guidelines defined by the Ministry of Culture and the Associazione Parchi e Giardini d'Italia. Villa De Capoa covers 16,000 m$^2$ and was transformed into an elegant Italian garden at the end of the 19th century adding numerous flowerbeds with a geometric design and some with more creative ones, all of which are surrounded by *Buxus sempervirens* L. hedges. It is located on the edge of the center of the "garden city" of Campobasso in an area that slopes towards the peri-urban area and overlooks the hills of Mount Vairano. The first steps of the project involved an archival, documentary, and bibliographic research and the inventory of the tree and shrub species present in the villa and the creation of the map of the distribution of the species. 181 angiosperms, representing 32 taxa, and 169 gymnosperms, representing 13 taxa, were included in the total number of individuals. Because of their longevity, rarity, and size, some of these species are considered valuable. Four individuals of *Sequoia sempervirens* (D.Don) Endl. (Endangered in IUCN) [1] tall about 30 cm and with a diameter around 115 cm, 4 individuals of *Cedrus atlantica* (Endl.) G.Manetti ex Carrière (Endangered IUCN) tall about 27 m and with a diameter around 54 cm, *Chamaecyparis lawsoniana* (A.Murray) Parl. (Near Threatened IUCN); one individual of *Platycladus orientalis* (L.) Franco (=*Thuja orientalis* L.) (Near Threatened IUCN); 17 individuals of *Aesculus hippocastanum* L. (Near Threatened IUCN). Other valuable species belong to the autochthonous regional flora are: *Quercus pubescens* Willd., *Quercus ilex* L. and *Fagus sylvatica* L. Among them, *Quercus pubescens* is 25 m tall and 90 cm in diameter, a fair number of seedlings are present for both oaks. In collaboration with forestry experts, a dendrometric investigation is being done to determine the precise date of the specimens. Species to be eradicated include mature individuals and/or suckers of *Ailanthus altissima* (Mill.) Swingle, *Robinia pseudacacia* L., *Prunus laurocerasus* L. which are considered very competitive invasive species. The results of the research will be exploited to create a story telling on the history of the garden, its transformation over time and on plant species of interest, dedicated to a vast public and, in particular, to the visually impaired.

Fig.1. Two individual of *Sequoia sempervirens* in D sector.

Fig. 2. *Prunus laurocerasus* suckers (invasive species).

1) https://www.iucnredlist.org/ Accessed on 21 June 2023.

## 5.5 = Botanical assessment of plants, fruits, and flowers in funerary paintings in Etruscan Necropolis of Monterozzi (Tarquinia) (UNESCO Site)

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In ancient times the representations of human fact, symbolism, and allegory were predominant, and the naturalist elements were chosen with a specific meaning. It can also be supposed for the funerary paintings at Tarquinia, which have an extraordinary cultural and artistic relevance, also describing the Etruscan landscape and environment. The relationship between humans and nature is well described in these paintings that represent the view of life, such as the funeral rites and the concept of the afterlife of the Etruscan aristocracy (1,3). Here we analyse the floristic biodiversity, such as the frequency and recurrence data, but overall, the iconographic value of the wide and neglected plant elements in the Etruscan paintings of Necropolis of Monterozzi in Tarquinia. Then, between 2021 and 2023, we analysed thirty-four Etruscan tombs within the necropolis and for each tomb, we photographed the various plant elements and the context in which they are present, and then we created a dataset in which the collected data were analysed. Thereafter, we proceeded with careful bibliographic research, and with the consultation of ancient texts. Regarding plant identification, we used different iconographic sources such as Dryades, but also Plant List (2003), World Flora Online, and the Italian Flora of Pignatti (2016). Our results show that most of the analysed tombs presents natural elements, and overall plant representations, such as fruits and flowers (e.g., *Punica granatum*, *Vitis vinifera,* and *Nymphaea lotus*), with augural intent for the funerary context. Some of them are combined in crowns and other represented in single elements. Many paintings also contain trees or entire herbaceous plants, having different evident symbolic elements. Very common is the presence of laurels (*Laurus nobilis* L.) and myrtles (*Myrtus communis* L.), for example, in the tomb of Hunting and Fishing. Also, spruces (*Picea* and *Abies*) and cypresses (*Cupressus sempervirens* L.), with an evident symbolic value, are particularly recurrent and contribute to making a frame and/or setting the representations. The role of some plants, such as *Verbascum* sp., *Euphorbia* sp., *Phyllitis scolopendrium*, *Papaver* sp., have an evident connection with the transcendent meanings of the object of representation itself. Some of these elements are autochthonous species, representing the ancient landscape of the Etruscan time, whereas others come from the Middle East. Such elements also describe the connections with the ancestors and their wide commercial routes. These preliminary results are intended as a starting point for broader studies of Etruscan art to systematically contribute to our knowledge of their culture. Recognizing the symbolic interpretation of plant species will be fundamental to understanding the link between Etruscan civilization and nature and landscape.

1) S. Steingraber (2006) Abundance of life: Etruscan wall painting. Getty Publications.
2) J.R. Jannot (2009) The Lotus, Poppy and other Plants in Etruscan Funerary Contexts.". Etruscan by Definition: The Cultural, Regional and Personal Identity of the Etruscans. Papers in Honour of Sibylle Haynes, 81-86.
3) M. Marzullo (2016) Grotte Cornetane: Materiali e apparato critico per lo studio delle tombe dipinte di Tarquinia. Grotte cornetane.

## 5.5 = Ethnobotanical survey as a tool to evaluate a potential ecosystem service in an urban park in Rimini (Emilia-Romagna, Italy)

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With the increase in the world population and the expansion of urbanized areas, greenspaces have gained importance as places where people get in touch with nature, which has been demonstrated to be beneficial for human health and well-being (1). In addition, greenspaces play a fundamental role in providing a "refuge area" for plant and animal species, thus helping to maintain a certain level of biodiversity within anthropized areas. A recent survey carried out on green areas in Berlin has demonstrated how they also contribute to keeping the ethnobotanical knowledge alive in the population thanks to a "transgenerational" heritage (2).
The present project aims to gather data about the knowledge of the use of plants and the level of awareness of urban biodiversity, through an ethnobotanical survey carried out in an urban green area. Data were gathered, between October 2021 and May 2023, through a *citizen science* approach, by means of semi-structured interviews that were conducted by a group of 38 high-school students to citizens attending one of the main green parks in Rimini (Emilia-Romagna, Italy). All the students involved in this project collected the data in the framework of the Ministry of Education mandatory program "Percorsi per le Competenze Trasversali e l'Orientamento".
The drafted interview guide included both open-ended and closed-ended questions and was divided into 4 sections: (A) background, (B) gathered plants, (C) personal information (D) univocal code based on personal information. From these semi-structured interviews, 422 citations were obtained from 176 informants, aged in the range 5-91 years. The informants, 111 females and 65 males, reported information on 108 different plant species belonging to 47 different plant families. All the 422 citations were used to build a data matrix for the data analysis.
As concerns the exploitation of this urban green area, 53% of informants was favorable to the use of this space to gather plants for different purposes, while 31% disliked the gathering practice in these areas, and the majority of this second group considers these urban green areas to be polluted and dirty, hence not suitable for gathering. The remaining 16% did not answer.
Regarding how knowledge about plants was acquired, 66% of the informants declared that they learned how to use plants thanks to an oral-transferred knowledge and 37% learned by themselves thanks to personal interests. Only a minority (2%) learned about this activity through private courses.
The main category of use, with a 59% of citation, is the food one. In this category, several plant belonging to the culinary tradition of this region were mentioned and used to prepare local dishes like piadine, cassoni and pasta.
To conclude, the Ausa urban park in Rimini results to be an important focus point for people who gather plants in this city, thus shown to play a crucial role for the community and to offer a relevant ecosystem service.

1) P.A. Sandifer, A.E. Sutton-Grier, B.P. Ward (2015) Ecosystem Services, 12, 1-15.
2) J.L. Landor-Yamagata, I. Kowarik, L.K. Fischer (2018) Sustainability, 10(6), 1873.

## 5.5 = Role of historic gardens in natural heritage conservation strategy: the example of the Giardino Inglese of Reggia di Caserta.

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The Giardino Inglese of the Reggia di Caserta is among the most important and ancient historic gardens in Italy[1]. As historic garden, it is a complex cultural testimony of aesthetic, scientific and technological interests that, throughout the life of the garden, have contributed to its creation and maintenance/modification. The preservation of a historic garden appears intimately connoted by a precise need for active intervention, based on the understanding of that environment as a cultural product. Preserving a historic garden may therefore mean maintaining or recovering a vegetation that respects the original or, more often, series of variations that have overlapped over the centuries and that have determined the current overall shape of the garden[2]. In this work, a survey of vegetation was carried out, and a collection catalogue was produced using updated nomenclature and Angiosperm Phylogeny Group (APG) IV criteria classification. The data from survey were georeferenced in a GIS environment. The evolution of the collection through time has been assessed searching data in the historical archives and recording the data of introduction of the specimens in the Giardino Inglese of the Reggia di Caserta. Of all the species surveyed, about half were not found in historical records regarding their introduction to the garden, but there are individuals of these species in the park that on the appearance could be candidates as monumental trees following extensive analysis to estimate their age. For each species, the conservation status at world level as per International Union for Conservation of Nature (IUCN) and Botanic Gardens Conservation International (BGCI), the phytosanitary status, and the number of individuals in the collections were evaluated. Two synthetic indices were created that allow the evaluation of collection's erosion risk (ER)[3]. In the analysis, each species is placed in a specific quadrant indicating its level of erosion risk, and a priority score (PS) allows the evaluation of the priorities of any conservations interventions[3]. The collection consists of 235 species, and approximately 65% of these are reported in IUCN Red List. 1671 individuals were registered. The database and the ER assessment allows the future development of tools for the management of the site. The Giardino Inglese is a garden of collections and a conservation basin for historical and monumental specimens of many species, some of which are subject to protection. The innovative method proposed in this work turns out to be quick and synthetic. Considering the botanical collection as an open-air museum, it becomes vitally important to have a precise picture of the state of health of the "works of art" present. The use of the erosion risk assessment allows the identification of species that are potentially at risk and in need of monitoring, to preserve the integrity of the botanical collections. The use of the PS, integrated with the ER, demonstrated to be a valid tool with great potential in the management of collections and in the evaluation of the allocation of resources for the protection of historic gardens[3].

1) C. Guarino, F. Napolitano (2001) The plants of "English garden" in the Royal Park of Caserta (S Italy). Quad Bot Ambientale Appl., 12, 45–66.
2) https://doi.org/10.1016/j.biocon.2016.11.005
3) https://doi.org/10.1080/11263504.2020.1810812

## 5.5 = Valorization of natural history collections through digitization: the Vatova-Schiffner's algarium

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The information stored in Natural History Collections (NHCs) are pivotal for a wide array of research activities. The mobilization of NHCs specimens by means of digitization - their conversion into accessible digital content - can greatly increase the fitness for use of NHCs across multiple disciplines. By enhancing the accessibility of specimen data under common standards and harnessing the power of distributed datasets, digitization can contribute to building new knowledge based on falsifiable scientific data, and - at the same time - increasing natural history heritage value to society.
This contribution aims at showcasing the digitization project (1) of the algarium of Aristocle Vatova and Victor Schiffner, which has been carried out by the Natural History Museum of Venice Giancarlo Ligabue, Fondazione Musei Civici di Venezia, together with the Department of Life Sciences, University of Trieste, also in the framework of the digitization activities of the National Biodiversity Future Center (NBCF). The project was developed and carried out as a replicable best practice for the digitization of historical NHCs.
The algarium is preserved at the Natural History Museum of Venice and consists of 1406 sheets. The specimens were collected from 70 monitoring sites in the Venice Lagoon between 1930 and 1932, and were used for writing the chapter dedicated to algae in the monograph "La Laguna di Venezia" published in 1938 (2). Therefore, the collection offers a detailed snapshot of the algal community of the Lagoon at the time. Its specimens' data can thus be pivotal for understanding the evolution of Lagoon's biodiversity in the last century.
Since until now this collection was almost unknown, the digitization project aimed at enhancing its usability and accessibility for the scientific community, as well as at making it available to citizens, along with information on the historical framework of the Lagoon in those years. Hence, the collection has been exploited both for research and conservation purposes, as well as for lifelong learning and virtual exhibits.
A suitable digitization workflow was developed on the basis of best practices and examples from literature. Different approaches together with major impediments to historical collection's digitization were investigated, taking into account every step, from digital imaging and transcription of specimen labels to geo-referencing, data management and publication.
Furthermore, since the project aimed at formulating novel approaches for the valorization and reuse of digitized products, rules and licenses for the publication of data in open and FAIR data formats were investigated, together with the better approaches for their exhibition to researchers and the general public.
Thanks to this effort, the collection is now available online in a web portal (https://dryades.units.it/MUVE_VS/index.php). Replicable valorization strategies that encompass attractive dissemination and production of educational materials such as leaflets, postcards, and photographic exhibition were also implemented.
This contribution provides an overview of the planning and execution of the digitization project, as a baseline for developing novel best practices and guidelines for facilitating digitization, curation, and data linking of NHCs among natural history museums in national and sovra-national networks. The outcome is an overall enhancement of the value of physical specimens, and of the fitness for use of their metadata, by creating new layers of data, empowering a global community, and developing novel approaches for conservation.

1) R. Trabucco, L. Seggi, S. Armeli Minicante, S. Martellos (accepted, in press) Un secolo di cambiamenti nella Laguna di Venezia attraverso le alghe della collezione Vatova-Schiffner. In: Atti del XXXI Congresso ANMS, "Musei scientifici, ambiente, territorio. Nuove visioni, obiettivi, servizi, relazioni per comunità sostenibili". Aosta, 18-21 ottobre 2022. Museologia Scientifica Memorie, 23.
2) V. Schiffner, A. Vatova (1938) Le Alghe della Laguna di Venezia. Sezione I. Chlorophyceae, Phaeophyceae, Rhodophyceae (Appendix: Myxophyceae). In: Brunelli G., Magrini G., Miliani L., Orsi P. (eds.) La Laguna di Venezia: monografia. Vol. III, Parte V, Tomo IX, Fasc. I. Tip. Carlo Ferrari, Venezia, 83-250.

## 5.6 = *Illicium verum* essential oil: GC/MS profile, quorum sensing and biofilm inhibitor on foodborne bacteria

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Nowadays, the overuse of antibiotics to treat infectious diseases contributed to the emergence of multidrug-resistant bacterial strains (1). For this reason, the food industry often tries to use natural resources such as essential oils in the preparation of foods (2). Medicinal and aromatic plants are a rich reservoir of bioactive molecules, able to promote health and to be used as drugs (3). Essential oils and plant extract represent an alternative to synthetic antioxidants and antimicrobial agents in food industry, as well as in pharmaceutical industry, alternative medicine, and natural therapy. *Illicium verum* Hook. f. (Illiciaceae) or star anise has many uses, ranging from culinary to religious ones. It is used in since ancient times for its high number of phytochemicals that are characterized by a large spectrum of biological activities. The main aims of this study were to determine the chemical composition and to evaluate the possible antibacterial, antibiofilm, and anti-quorum sensing activities of the essential oil (EO) obtained by hydro-distillation of its aerial parts. Twenty-four components were identified representing 92.5% of the total. (E)-anethole (83.7%), limonene (3.2%), and α- pinene (0.7%) were the main constituents of the EO. Results showed that the EO was effective against eight bacterial strains (*Listeria monocytogenes, Vibrio vulnificus*, *Salmonella enterica*, *Shigella flexeneri*, *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*) with different degree. *trans*-Anethole showed more potent antibacterial effects when compared to *I. verum* with inhibition zone ranging from 6.0 ± 0.1 to 11.7 ± 0.6 mm. The Minimum Inhibitory Concentrations (MICs) of both *I. verum* EO and *trans*-anethole were about 0.048 mg/mL for each bacterial strain; the Minimal Bactericidal Concentrations (MBCs) were >50 mg/mL. Concerning the antibiofilm activity, *trans*-anethole was more effective against biofilm formation than the whole EO when tested using sub-inhibitory concentrations. Results confirm the alternative of use of this plant to treat human diseases because of its effectiveness and safety. Moreover, *I. verum* EO is a considerable natural antibacterial agent and might be used as a natural preservative in food industries.

1) C. Llor, L. Bjerrum. (2014) Ther Adv Drug Saf., 5, 229-241.
2) B. Outemsaa, A. Oubihi, H. Jaber, S. Haida, I. Kenfaoui, R. Ihamdan, H. El Azhari, M. Ouhssine. (2021) E3S Web of Conferences, 319(1), 1-7.
3) M. Aminzare, M. Hashemi, H.H Azar, J. Hejazi. (2016) J. Hum. Environ. Health Promot., 1, 63–74.

## 5.6 = Different types and doses of ionizing radiation influence the morpho-anatomical and phytochemical traits of *Brassica rapa* microgreens

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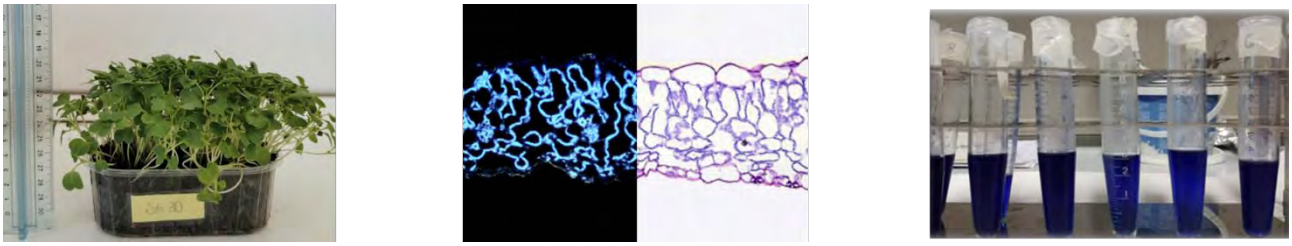
For the success of Moon and Mars colonization by humans, astronauts will need to be self-sufficient regards the regeneration of essential resources, such as oxygen, water, and food. Compared to other photosynthetic organisms, higher plants are essential in this context since, apart to resources regeneration, they provide a broad range of fresh food rich in bioactive compounds. Such molecules may improve and empower their resistance against space-induced diseases. However, plant cultivation in space must overcome several issues due to the unfavorable environmental factors, such as ionizing radiation, that may limit the cultivation reducing plant yield and nutritional quality. Plants at different phenological phases are characterized by different radio-resistance, and the actively growing meristems are more sensitive compared to dormant seeds.

The purpose of this study was to analyze the effect of different ionizing radiation (IR) quality and dose on morpho-anatomical and nutritional traits of microgreens of *Brassica rapa* L. subsp. *sylvestris* var. *esculenta*. Germinated seeds were exposed to X-rays and $^{56}$Fe ions beams delivered at increasing doses (0-control, 0.3, 1, 10, 20, and 30 Gy for X-rays; 0-control, 0.3, 1, 10, 20, and 25 Gy for $^{56}$Fe ions).

After irradiation, microgreens were cultivated under controlled conditions, and morpho-biometric traits (e.g., leaf area, fresh and dry biomass), were measured at the harvest (Fig. 1a). Leaf anatomical traits (e.g., lamina tissues thickness and stomatal frequency) were quantified through light and epi-fluorescence microscopy and digital image analysis (Fig. 1b). The above-ground biomass was analyzed in terms of chlorophyll content and total antioxidant capacity (Fig. 1c).

The results indicate that the response of *B. rapa* was strictly related to the analyzed trait, and type of ionizing radiation and dose delivered. This information is useful in clarifying the mechanisms of the specie-specific radioresistance fundamental in the choice of crop candidate for the cultivation in space.



(a) (b) (c)

Fig. 1: Microgreens of *Brassica rapa* L. during: morpho-biometric measurements (a); light and epi-fluorescence microscopy view of the leaf lamina (b); quantification of antioxidant capacity (c).

## 5.6 = Wood distillate boosts antioxidant properties of *Solanum lycopersicum*

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Bio-stimulants may be valuable substitutes of chemical fertilizers to boost the productivity and quality of crops. Different bio-based products are currenlty now on the advertise; one of the most up to date and most promising is wood distillate (WD), which may be a byproduct of the pyrolysis of plant biomass from waste forest timber utilized for bio-energy generation (1).
Wood distillate is wealthy of biologically active compounds such as polyphenols, moving forward plant wellness and surrender execution. Its foliar application has been broadly studied and comes about have appeared to highlight the antioxidant profile of developed crops (2).
In this context, WD has been utilized as foliar added substance for *Solanum lycopersicum* L. Its application improved fresh weight (+16.0%), soluble sugars content (*i.e*., glucose (+32.9%), fructose (+24.4%)), and add up to (+27.8%) antioxidant pool content (*i.e*., polyphenols (+17.9%), flavonoids (+58.1%)), and lycopene substance (+51.9%).
No significant contrast within the mineral component has been observed between controls and the WD-treated tomato natural products, but for the phosphorus, which appeared a noteworthy lessening in WD-treated tomatoes by 24.1%.
The two tomatoes have been subjected to extraction by means of acetone/water blend for advance chemical characterization. The $^{1}$H-NMR and ESI-MS analyses of the extracts revealed the presence of different fatty acids, but also amino acids and sugars. In particular, the WD-treated tomato showed the presence of pyroglutamic acid and phloridzin derivatives, but also dihydrokaempferol, naringenin glucoside, cinnamic acid and kaempferol-3-O-glucoside.
These results clearly show the efficacy of using WD in improving the yield and nutritional qualities of edible parts of crops. Further analyses will be able to evaluate in cellular studies the antioxidant profile emerged *in vitro*.

1) A. Grewal, L. Abbey, L.R. Gunupuru (2018) Journal of Analytical and Applied Pyrolysis, 135, 152-159.
2) R. Fedeli, A. Vannini, S. Celletti, V. Maresca, S. Munzi, C. Cruz, D. Alexandrov, S. Loppi (2023). Annals of Applied Biology, 182(1), 57-64.

## 5.6 = Nutraceutical properties of *Chenopodium quinoa* seeds and sprouts under different experimental conditions: response to saline stress and biostimulant agents

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The cultivation of quinoa (*Chenopodium quinoa* Willd.) has increased in recent years as food rich in nutritional and nutraceutical properties, naturally gluten-free, source of protein and essential amino acids, micronutrients and antioxidants, suitable for celiac and vegetarian/vegan diets. The quinoa plant resistance to various environmental stresses allows its cultivation in extreme environments but could affect its nutritional quality (1).
The aim of this research was to characterize the content of antioxidant substances and total antioxidant capacity in the first Italian variety of quinoa called “quipu”, in particular on raw, cooked and fermented seeds. It was also analyzed how saline stress (NaCl 100, 200, 300mM) in the presence or absence of biostimulant microorganisms (endophytic bacteria from seeds or *Chlorella* sp. microalgae), can influence the germination and the concentration of antioxidant substances in seeds and sprouts. Results indicated that the concentration of bioactive molecules did not change after cooking the seed, while it increased after fermentation.
After salt stress a general dose-dependent increase of antioxidant molecules (polyphenols, flavonoids, chlorophylls, carotenoids, anthocyanins) and activity (determined by FRAP, Ferric Reducing Antioxidant Power, and DPPH, 2,2-diphenyl-1-picrylhydrazyl assays) was observed.
Salt stress in the presence of biostimulant agents had different effects on the growth of quinoa seedlings and on their content of antioxidant molecules.
In conclusion, natural treatments such as the fermentation or the use of specific biostimulants under saline stress conditions, can improve plant growth and increase the concentration of bioactive molecules and antioxidant activity. These results may be the basis for future research on the cultivation of quinoa in marginal lands and on the use of quinoa extracts to formulate food supplements with antioxidant properties.

1) A. Souid, L. Bellani, E.L. Tassi, K. Ben Hamed, V. Longo, L. Giorgetti (2023) Antioxidants, 12, 1060.

## 5.6 = Protective role of an extract waste product from *Citrus bergamia* in an in vitro model of neurodegeneration

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A balanced diet, rich in fruits and vegetables and ensuring the intake of natural products, has been shown to reduce or prevent the occurrence of many chronic diseases. However, the choice to consume large quantities of fruits and vegetables leads to an increase on the amount of waste, which can cause an alteration in environmental sustainability. To date, the concept of a "byproduct" has evolved, now being understood as a waste product from which it is still possible obtain useful compounds. Byproducts in the agricultural sector are a rich source of bioactive compounds, capable of possessing a second life, decreasing the amount of waste products, the disposal costs, and environmental pollution.
A promising and well-known citrus of the Mediterranean diet is the bergamot (*Citrus bergamia*, Risso et Poiteau). The composition of bergamot is known, and the rich presence of phenolic compounds and essential oils has justified the countless beneficial properties found, including anti-inflammatory, antioxidant, anti-cholesterolemic, and protective activity for the immune system, heart failure, and coronary heart diseases. The industrial processing of bergamot fruits leads to the formation of bergamot juice and bergamot oil. The solid residues, referred to as "pastazzo", are normally used as feed for livestock or pectin production. The fiber of bergamot (BF) can be obtained from pastazzo and could exert an interesting effect thanks to its content of polyphenols. The aims of this work were twofold: (a) to have more information (composition, polyphenol and flavonoid content, antioxidant activity, etc.) on BF powder and (b) to verify the effects of BF on an *in vitro* model of neurotoxicity induced by treatment with amyloid beta protein (Aβ). In particular, a study of cell lines was carried out on both neurons and oligodendrocytes, to measure the involvement of the glia and compare it with that of the neurons.
The results obtained showed that BF powder contains polyphenols and flavonoids and that it is able to exercise an antioxidant property. Moreover, BF exerts a protective action on the damage induced by treatment with Aβ, and this defense is found in experiments on the cell viability, on the accumulation of reactive oxygen species, on the involvement of the expression of caspase-3, and on necrotic or apoptotic death. In all these results, oligodendrocytes were always more sensitive and fragile than neurons. Further experiments are needed, and if this trend is confirmed, BF could be used in AD; at the same time, it could help to avoid the accumulation of waste products.

## 5.6 = Flowers of the genus *Oxalis* as a new source of healthy compounds

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Edible flowers are an integral part of culinary traditions in many cultures, including ours. Scientific research has highlighted their rich content of health-beneficial molecules such as sugars, antioxidants, vitamins, and minerals, especially when consumed fresh. Our study is part of Italy France INTERREG ALCOTRA bilateral European project "ANTES" (n. 8336), which aims to extend the consumption of edible flowers as functional foods by increasing the variety of species used for the supply chain. Plants of the genus *Oxalis* are common Mediterranean weeds, exhibiting significant variability among the species present in Italy, each with its preferred habitat. *Oxalis pes caprae* L. and *Oxalis articulata* Savigny plants are collected during early spring when new shoots appear and the young leaves are most palatable (1). The leaves are already widely used in culinary traditions, particularly in salads, due to their sour and salty taste, which makes them very refreshing. *O. pes caprae* leaves contain a good amount of phenolics with antioxidant activity (2).
In this study, flowers of both species were collected in two different private gardens (Pisa district and Elba Island) and freeze-dried for long-term storage. The freeze-dried flowers were used to investigate the nutraceutical properties including carotenoids, polyphenols, flavonoids, anthocyanins, ascorbic acid, and sugars, through spectrophotometric analysis. Additionally, antioxidant activity was determined (ABTS and DPPH assay). Both species demonstrated moderate quantities of nutraceutical compounds, even when compared to other edible flowers reported in the literature (3). *O. pes caprae* L. proved to be a good source of polyphenols and flavonoids, displaying notable antioxidant activity. Anthocyanins were exclusively detected in *O. pes caprae*. It is well-established in the literature that *Oxalis* leaves contain a good quantity of vitamin C (1), and the flowers of both species confirm the presence of an excellent quantity of Vitamin C. Soluble sugars were found to be similar across the samples. In the aroma profile analysis conducted using HS-SPME and GC-MS the flowers of *O. pes caprae* flowers were found to have a notable presence of sesquiterpene hydrocarbons, with β-caryophyllene being the prominent compound. On the other hand, the volatile fraction of *O. articulata* was characterized by non-terpene derivatives especially *n*-nonanal and methyl salicylate, followed by sesquiterpene hydrocarbons such as α-humulene.
Therefore, owing to their phytochemical content and ubiquitous presence as weeds, plants of the genus *Oxalis*, constitute an easily accessible and cost-effective source of bioactive molecules that could be incorporated into daily diets as food supplement.

Fig.1. *Oxalis articulata* Savigny.

Fig.2. *Oxalis pes caprae* L.

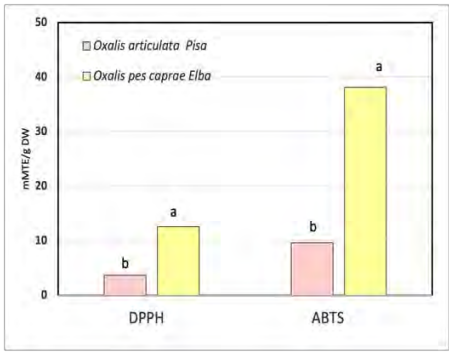


Fig.3. Antioxidant activity of flowers (n=5; Anova Past3, withTuckey HDS).

1) H. Šircelj, M. Mikulič-Petkovšek, F. Batič (2010) Food Chemistry, 123(2), 351-357.
2) M.C. Gaspar, D.A. Fonseca, M.J. Antunes, C. Frigerio, N.G. Gomes, M. Vieira, A.E. Santos, M.T. Cruz, M.D. Cotrim, M.G. Campos (2018) Natural product research, 32(6), 732-738.
3) I. Marchioni, B. Najar, B. Ruffoni, A. Copetta, Lu. Pistelli, La. Pistelli (2020), Plants, 9(6), 69.1

## 5.6 = Influence of pedoclimatic conditions and harvest time on *Pisum sativum* 'Eso' seed phytochemical profile

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Yellow pea (*Pisum sativum* L. sin. *Lathyrus oleracaeus* Lam. 'Eso'*)* is an annual herbaceous plant that belongs to the *Fabaceae* family. It generally grows in cold climates and at high altitudes. Many cultivars tend to ripen 60 days after sowing. Given climatic needs, consumption and associated costs, they are commonly found as a subsistence crop in many Third World countries, or as a mechanized crop for animal feed or canning. Peas, along with other legumes, are an excellent source of protein and essential amino acids; the yellow variety in particular is known for maintaining a good nutritional profile even if subjected to industrial processing (1). Therefore, they are the subject of numerous studies aimed at defining new protein sources. However, the presence of antinutrients such as phytate is known, which reduces the absorption of mineral salts and oligosaccharides which reach the intestine without being metabolized and which act as a substrate for the methanogenic bacteria of the intestinal bacterial flora with consequent flatulence problems (2).
The aim of the study is therefore to evaluate the changes in the entire phytochemical profile of yellow pea seeds as a function of both the place of cultivation (Pianura Padana compared to Altopiano del Fucino) and as a function of the harvest time. The method used is high-resolution NMR spectroscopy, which allows the qualitative and quantitative evaluation of all the molecular classes present in a sample with a single experiment without having to resort to standards structurally similar to these molecules.
Commercial seeds from the Po Valley area were analysed. Yellow peas were planted in the Fucino plain by Aureli Mario Agricola company. The seeds were collected at about 40, 50, 60 and 70 days from sowing (n=6 for each sampling), the last point representing full maturity and then comparable to commercially available seeds. A chloroform:methanol:water 2:2:1.5 multisolvent extraction procedure was performed on each sample and the hydroalcoholic fractions were examined by high-resolution NMR spectroscopy employing the Jeol JNM-ECZ 600R spectroscope available to NMLab (3).
40 molecular species have been identified and quantified: amino acids (Alanine, Asparagine, Aspartic Acid, Arginine, γ-Aminobutyric acid, Glutamine, Glutamate, Histidine, Leucine, Isoleucine, Lysine, Phenylalanine, Threonine, Tryptophan, Tyrosine, Valine), organic acids (Acetate, Citrate, Formate, Fumarate, Malate, Succinate), carbohydrates (Sucrose, Galactose, Glucose, Xylose, Fructose), oligosaccharides (Raffinose, Stachyose, Verbascose), secondary metabolites (Hydroxycinnamic Acid, Indole-3-Acetate, Caffeic Acid, 4-Hydroxyphenylacetate, Trigonelline, Resveratrol Glucoside) and other compounds (Phytate, Choline, Adenosine, Guanosine, Uridine).
From the univariate and multivariate statistical analyses conducted on the examined matrices, it was observed that there is a monotonous increase in carbohydrates and a monotonous decrease in amino acids as a function of harvest time. Oligosaccharides, phytate and nitrogenous base levels increased, but only in later sampling times. As far as the pedoclimatic comparison is concerned, the yellow peas collected in the Fucino area showed higher values of secondary metabolites (mainly 4-Hydroxycinnamic acid and Resveratrol Glucoside) and aspartate, while in the ones of the Po Valley, Citrate and Succinate were higher, as well as Glutamine and Asparagine levels. These metabolites can be associated with mechanisms of response to stress due to temperature conditions and amount of precipitation.
This study adds new information to outline the best moment of seed collection to optimize the content of amino acids and secondary metabolites with that of antinutrients for the best nutritional outcome for humans.

1) S.L. Wray, S. Cenkowski (2002) Transactions of the ASAE, 45, 1023-1027.
2) M. Horbowicz, R.L. Obendorf (1994) Seed Sci Res, 4, 385-405.
3) O. Giampaoli, F. Sciubba, G. Conta, G. Capuani, A. Tomassini, G. Giorgi, E. Brasili, W. Aureli, A. Miccheli (2021) Foods, 10, n°1877.

## 5.7 = Evaluation of the antioxidant properties of fractions from the flower hydroalcoholic extract of *Sinapis pubescens* subsp. *pubescens* (Brassicaceae) grown wild in Sicily (Italy)

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In the last decades, plants that grow spontaneously have aroused remarkable interest for their considerable importance in the agri-food sector and for the treatment of many diseases. The taxa included in the *Brassicaceae* family, well described in the recent scientific literature and widely cultivated in the Mediterranean regions, are extremely important for their economic and nutritional value and result to be attractive sources of nutraceutical compounds. As part of a project aimed at the valorization of taxa included in the *Brassicaceae* family belonging to the spontaneous flora of Sicily (Italy), recently, our research team has designed a study on the specific and intraspecific taxa of *Sinapis* L., utilized in traditional medicine and as food. Particularly, current research is focused on *Sinapis pubescens* L. subsp. *pubescens* (hairy mustard), an edible species not studied so far.
*Sinapis pubescens* subsp. *pubescens* is a perennial herbaceous plant, with pubescent or villous stems, generally between 30 and 80 cm tall. The leaves are pubescent; the lower ones are stalked, lyrate-pinnatisect, and the upper ones are sessile, oblong, less divided or simple. The flowers, gathered in racemes, have yellow-greenish sepals and yellow spatulate petals. The fruit is a siliqua with curved beak. In many Italian regions, including Sicily, the aerial parts of *S. pubescens* subsp. *pubescens* (tender shoots, leaves, and inflorescences) are used for the preparation of soups, in salads, boiled or fried. The above statements prompted our group to design a study aimed at investigating the biological properties of the aerial parts (flowers, leaves, and stems) of *S. pubescens* subsp. *pubescens* in relation to their phytochemical profile.
In our previous work, the phenolic profile and the antioxidant potential of *S. pubescens* subsp. *pubescens* flower hydroalcoholic extract (70% MeOH) were investigated. Specifically, the phenolic constituents of the extract were determined spectrophotometrically and characterized by HPLC-PDA/ESI-MS analysis, which led to the identification of 26 flavonoids and 2 phenolic acids. The antioxidant properties were studied by *in vitro* methods based on different mechanisms: the primary antioxidant activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reducing power assays, while the secondary antioxidant properties were determined by the ferrous ion chelating activity assay. The flowers of *S. pubescens* subsp. *pubescens* were found to be a valuable source of antioxidant compounds, exhibiting both primary and secondary antioxidant properties. In the present work, in order to clarify the effective role of the constituents of the phytocomplex in determining the different antioxidant mechanisms, the crude extract was suspended in distilled water and subsequently fractionated by liquid-liquid extraction with organic solvents of increasing polarity: *n*-hexane (*Spp*-HEX), dichloromethane (*Spp*-DCM), *n*-butanol (*Spp*-nBA) and ethyl acetate (*Spp*-ETAC). Then, the fractions were evaluated for their total phenolic content and *in vitro* antioxidant properties. The Folin-Ciocâlteu colorimetric assay highlighted the predominance of phenolic compounds in the *Spp*-ETAC fraction (310.296 ± 2.071 mg GAE/g), followed by *Spp*-nBA (60.030 ± 2.126 mg GAE/g). The phenolic-rich *Spp*-ETAC fraction exhibited the strongest primary antioxidant properties in terms of radical scavenging activity ($IC_{50}$ = 0.097 ± 0.017) and reducing power (ASE/mL = 5.938 ± 2.430); a different trend was observed in the ferrous ion chelating assay, since the *Spp*-ETAC and *Spp*-nBA fractions did not show any activity, whereas it was highlighted for the *Spp*-HEX and *Spp*-DCM fractions ($IC_{50}$ = 0.724 ± 0.068 mg/mL and 0.159 ± 0.005 mg/mL, respectively). Such results suggest a limited contribution of the phenolics to the secondary antioxidant properties of *S. pubescens* subsp. *pubescens* flower hydroalcoholic extract, which seem to be determined by other classes of metabolites. Further studies are needed to characterize the fractions from *S. pubescens* subsp. *pubescens* flowers, which could represent a new potential source of natural antioxidants for the formulation of nutraceuticals and dietary supplements able to provide beneficial effects on human health.

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## 5.7 = Phytochemical profiles and biological activities of extracts rich in rosmarinic acid from *Origanum majorana* (Lamiaceae) flowers and twigs

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Nowadays, the ever-growing interest in natural products and eco-sustainability is pushing the research and the market toward the development of techniques with no environmental impact for the extraction of bioactive compounds of plant origin, also aiming to requalify waste products from food industry, converting them into raw materials for the production of health care products. Furthermore, the effectiveness of natural compounds and phytocomplexes in the prevention, treatment and/or control of numerous pathological conditions has been increasingly highlighted and demonstrated (1). In this prospective, we have optimized a green extraction technique to obtain extracts rich in rosmarinic acid from different parts of *Origanum majorana* L., in particular, leaves and flowers (used as spices) and twigs (waste material). *O. majorana* is a plant belonging to the Lamiaceae family distributed in many countries of the Mediterranean area, it's commonly used for food purpose, but it has also been widely employed in traditional medicine to treat fever, allergies, respiratory infections, and various forms of pain (2). *O. majorana* is particularly rich in rosmarinic acid (RA), a non-flavonoid phenolic compound typical of the plants belonging to the Lamiaceae family, whose health-promoting properties, like antioxidant, anti-inflammatory, antiviral, antibacterial, analgesic, hepatoprotective, immunomodulatory, anticancer, cardioprotective and neuroprotective, are widely known (3).
For the extraction, we used an hydroalcoholic solution (being ethanol considered a green solvent), testing different percentages of ethanol (30, 50, 75%, v/v), finding that the 50% solution gave the best absolute yield and the highest content of rosmarinic acid, in both leaves and flowers extract (MF50) and twigs extract (MT50). Interestingly, by HPLC-DAD analysis, we found that the MT50 extract contains more RA (2.97%) than MF50 (2.59%) and possesses a qualitatively and quantitatively richer profile, as confirmed by the Folin-Ciocalteu and Aluminium Chloride methods, which showed that MT50 contains both more phenolic compounds (MT50: 271.22±4.33 mg GAE/g of extract; MF50: 245.47±5.95 mg GAE/g of extract) and flavonoids (MT50: 218.79±3.27 mg CE/g of extract; MF50: 159.26±6.84 mg CE/g of extract). Then, we applied *in-vitro* cell free and *in-vitro* cellular models to test the antioxidant and anticancer activity of both the extracts, more specifically, we performed DPPH test ($IC_{50}$: MT50 31.72±2.78 μg/mL; MF50 30.11±3.46 μg/mL), SOD-like activity assay ($IC_{50}$: MT50 0.79±0.04 μg/mL; MF50 0.71±0.06 μg/mL) and Catalase-Like activity assay ($IC_{50}$: MT50 119.55±8.96 μg/mL; MF50 103.09±7.84 μg/mL) to evaluate the scavenging activity of the extracts towards free radicals and ROS, finding similar antioxidant properties for the two extracts. We also carried out MTT tests to assess their cytotoxic activity on CaCo-2 ($IC_{50}$ 72h: MT50 268.16±14.52 μg/mL; MF50 154.10±10.07 μg/mL) and MCF-7 ($IC_{50}$ 72h: MT50 917.95±23.21 μg/mL; MF50 624.63±12.24 μg/mL) cancer cell lines, which showed that MF50 was more active than MT50. We further investigated the mechanism of action on CaCo-2 cell line (being more sensitive to the treatments) with LDH release assay, which allowed us to exclude necrosis as main cell death mechanism, ROS evaluation and non-protein thiols quantification. We used HFF-1 ($IC_{50}$ 72h: N.D.) as normal control human cell line to establish the selectivity towards cancer cells.

Fig. 1. *Origanum majorana* L. (Lamiaceae).

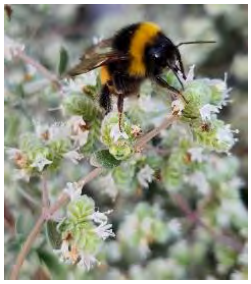

Fig. 2. Detail of *O. majorana* flowers.

1) E.R. Sauter (2020) Expert Rev Clin Pharmacol, 13(3), 265-285.
2) A. Bouyahya, I. Chamkhi, T. Benali, F.E. Guaouguaou, A. Balahbib, N. El Omari, D. Taha, O. Belmehdi, Z. Ghokhan, N. El Menyiy (2021) J Ethnopharmacol, 265,113318.
3) M. Hitl, N. Kladar, N. Gavarić, B. Božin (2021) Planta Med, 87(4), 273-282.

## 5.7 = The Role of botanical research in the framework of the International Cooperation: Unlocking the Potential of Wild Oil Plants in South Angola

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This study wants to outline the contribution of botanical research to the projects of international cooperation and development assistance, and what impact the botanical research may have on improving livelihood of poor people. In particular, this work explores the traditional uses of *Ximenia americana* L. and *Sclerocarya birrea* (A.Rich.) Hochst. by rural communities living in the arid areas of South Angola, with the general aim to promote these resources at national and international level and to enhance the economic conditions of local households. The specific aims are: (I) to examine the traditional production technique and (II) to investigate the chemical profile of the oils extracted from these species. A corollary objective of our study is to emphasize the importance of forest preservation and reforestation in the context of Non-Wood Forest Products (NWFPs) exploitation.

The research is conducted within the framework of the "Projecto Integrado de Resiliência Ambiental - Namibe (Integrated Environmental Resilience Project - Namibe)," implemented under the FRESAN program, funded by the European Union and managed by the Instituto Camões (Portugal). The aim of this initiative is to reduce the vulnerability of local communities, improving food reserves and local economic capacities through the enhancement of NWFPs in South Angola.

The oil extracted from the kernels of *Ximenia americana*, locally known as Mumpeke, exhibits valuable properties for cosmetic applications and could provide important opportunities in increasing family income.

*Sclerocarya birrea*, also known as Marula or Ngongo, is primarily utilized to produce an alcoholic beverage by fermenting its fruits; beyond this common traditional use, the research investigates the characteristics and importance of an edible oil extracted from the seeds, which is widely employed for household cooking purposes by local women. Understanding the ecological importance and the economic potential of these species can contribute to their sustainable management, while also generating income opportunities for the local population. Moreover, they can provide women with a possible independent income and status and contribute to their empowerment. Through academic research, international cooperation initiatives can validate project data, promoting effective sustainable resource management, community empowerment, and the preservation of traditional knowledge.

## 5.7 = Chemical characterization and evaluation of the antioxidant properties of *Anthemis parlatoreana* (Asteraceae)

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The investigations on the vascular flora of the coastal reliefs of North West Sicily allowed the identification of a new species named *Anthemis parlatoreana* Raimondo, Bajona, Spadaro & Di Grist. (Asteraceae) (1). In detail, it is a taxon belonging to the sect. *Hiorthia* and connected to *A. punctata* group that is also present in North Africa (2) and already represented in Sicily by two other endemic species, *A. cupaniana* Guss. and *A. pignattiorum* Guarino, Raimondo & Domina.
In the present study, *A. parlatoreana* fresh and dried aerial parts were subjected to distillation procedure for the extraction and isolation of essential oils. The volatile composition was characterized by gas chromatographic (GC) techniques. In both fresh and dried samples, the most abundant chemical classes were ketones and esters, represented to a greater extent by β-thujone and β-artemisia acetate, respectively. In dried vegetable material, a high content of α-pinene and δ3-carene was also quantified. Aqueous, methanol and ethyl acetate extracts of aerial parts were also investigated in terms of polyphenolic content by high-performance liquid chromatography coupled with photodiode array and electrospray ionization high resolution mass spectrometry (HPLC-PDA/ESI-MS). Moreover, the antioxidant potential of the extracts was also assayed. Results show that all the extracts evidenced a quite different quali-quantitative profile and in terms of chemical classes, most of compounds belonged to the hydroxycinnamic class followed by flavonoids, and hydroxybenzoic acid derivatives. Further, the dried methanolic followed by the dried aqueous extracts revealed the best quenching ability in the DPPH test, whereas concerning iron chelating activity, although all of them showed a marked capacity to disrupt or inhibit the formation of iron (II)-Ferrozine complex, a slight difference was observed between the dried and the fresh ones, since the former have a slightly higher chelating power than the latter.
In conclusion, on the basis of a previous study (3), a comparative picture between the chemical characters and the antioxidant properties of all the species of *Hiorthia* section studied to date in Sicily is here reported.

1) F.M. Raimondo, E. Bajona, V. Spadaro, E. Di Gristina (2021) Flora Medit., 31,109-122.
2) C. Oberprieler (1998) Bocconea, 9, 5-106.
3) L. Riccobono, A. Maggio, M. Bruno, V. Spadaro, F.M. Raimondo (2017) Natural Product Research, DOI: 10.1080/14786419.2017.1297444.

## 5.7 = Comparative study of the phytochemical composition of leaves and fruits of an Italian landrace of *Cyclanthera pedata*

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*Cyclanthera pedata* (L.) Schrad. is an annual herbaceous vine of Cucurbitaceae family native of the Andean territories of South America, where it is known as "Caigua" or "Achocha". The fruit is edible, and it is also considered a traditional herbal remedy. The aerial parts of the plant are used as well and the leaves are considered having medicinal properties. Caigua is largely cultivated in South America, but it was also introduced in various parts of the world, including Italy. This species adapted well to the climate of the Alpine Valleys, and in Camonica Valley the cultivation of a cultivar of Caigua called "Ciuenlai" is testified since 1960 (1).
Recently, Caigua has gained the interest of herbal industry for the preparation of food supplements or other health products (2). Different effects are attribute to this plant including anti-inflammatory, hypoglycaemic and hypocholesterolaemic activities. The main source of raw material for European herbal products manufacturer is the dried fruit imported from South America, but the Ciuenlai has proven to be a suitable alternative resource (1). To date, the fruit is the only part of the plant used in the production of herbal products, while leaves are considered a waste product. Caigua leaves are included in the list of Annex 1 of the Ministerial Decree of 10 August 2018, listing the plants allowed for herbal use, the preparation of extracts or other health products. However, the leaves are currently not used, although they represent most of the aboveground plant biomass.
Thus, the aim of this work was to evaluate the phytochemical profile of the Ciuenlai leaves in comparison with the fruit. Total phenols, flavones and flavonols, flavanone and dihydroflavonols and saponins content were quantified using spectrophotometric methods. The percentage caffeoylquinic acid content was also determined as described in the monograph of artichoke dry extract in Official Pharmacopoeia of the Italian Republic XII Edition. This method is used as parameter from the herbal industry to evaluate the quality of the batches of dried Caigua fruits. Additionally, a qualitative analysis of the HPLC profile of hydroalcoholic extracts has been performed to compare the phenolic profile of fruits and leaves.
The fruits showed a higher content of total phenols (29.66±0.63 mg GAE/g) and a similar content of flavones and flavonols (9.09±0.35 mg QE/g) than leaves (19.89±0.86 mg GAE/g and 7.76±0.46 mg QE/g). Conversely, the content of flavanone and dihydroflavonols (14.02±0.23 mg NGE/g) and the content of saponins (18.55±0.69 mg GSE/g) resulted higher in leaves. Additionally, the leaves showed a percentage caffeoylquinic acid content of the leaves (4.24±0.28 %) notably higher than fruits (0.64±0.04 %).
The HPLC profile of the leaves extract showed a large number of peaks, suggesting a more complex phenolic fraction than the one of fruits. A noteworthy aspect is the presence of the same chrysin derivative as major compound in both extracts.
These results show that the leaves of Ciuenlai have a rich content of secondary metabolites, including compounds (e.g., saponins) which are contained in lower amount in the fruits. Therefore, leaves could be an interesting source of active ingredients, also due to the large aerial biomass that the plant produces and that is usually considered a waste product.

1) M. Zuccolo, D. Pedrali, V. Leoni, G. Borgonovo, A. Bassoli, A. Giorgi, L. Giupponi (2023) Genetic Resources and Crop Evolution, 70, 1455.
2) J. Frigerio, E. Tedesco, F. Benetti, V. Insolia, G. Nicotra, V. Mezzasalma, S. Pagliari, M. Labra, L. Campone (2021) Frontiers in Pharmacology, 12, 726199.

## 5.7 = The Ethnomedicinal species of the Monte Pisano: from an ancient herbarium to today

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In 2005, an ancient *herbarium* called "of royal possession", concerning the flora of Pisa and some few plants of Lucca, Livorno, Grosseto and Firenze has been found in a storeroom of the San Rossore Estate within the Regional Park of Migliarino, San Rossore, Massaciuccoli (Pisa, Italy). It was drawn up at the end of 19$^{th}$ century (1883) by an anonymous author. The *herbarium* consists of 10 folders total, including 1529 sheets representing 1521 plant specimens in excellent condition (1). At the *herbarium* is attached an annotated catalogue that describes seven different types of botanical data: botanical names, common names, families, plant life form, flowering time, collection areas and the typical use of species. After a preliminary study (carried out in 2009), during which investigation was conducted on verify identification of the species and nomenclature updating, was counts 1248 species. From the ethnobotanical data analysis resulted that the highest number of species is characterized by an agropastoral use (66%), many species are mentioned for medicinal use (23%), handcraft use (10%) and foodstuff use (8%). The present study has focused on the vascular plants of the herbarium which show the indication "Monti Pisani" or the name of neighbouring localities as the collection area. The selected herbarium species were then compared with those reported in the most recent floristic lists of Monte Pisano to verify their current presence. The Monte Pisano is a relief of modest height (the highest peak is 917 m above sea level) but with this characteristic it can be considered a true mountain system that separate the plain of Pisa from the plain of Lucca, from North-West to South-East. This mountain, isolated in the middle of flat and marshy areas, has always played a significant role of source of livelihood and human settlements since Upper Palaeolithic era. Despite the significant role of Monte Pisano in the daily life of the rural population, we do not have updated information on the ethnobotanical use of plants in this area, and in particular on their medical use, which is the object of this study. Out of 1137 species recorded in the herbarium for the province of Pisa, 419 are specifically to be referred to the different areas of Monte Pisano. Of these, only 302 are confirmed in the most recent and complete floristic list (2) for the vascular flora of Monte Pisano. The *herbarium* attachment reported the medical use for 101 species, 87 of which are confirmed. The comparison with the most recent ethnobotanical literature of Tuscany, and in particular of the Monte Pisano area, has shown that only 24 of the cited 101 *herbarium* species were effectively with a confirmed popular ethnomedical use. In addition, some ethnomedical species recorded in the herbarium (the exact number is being processed) and confirmed by the literature are no longer present to date, as *Ruta graveolens* L., *Aristolochia pallida* Willd., *Cynoglossum officinale* L. and *Euphrasia stricta* D. Wollf. It is also interesting to note the presence in the herbarium of 13 species which have not been confirmed in the recent floristic lists and whose ethnomedical use is not confirmed in the local ethnobotanical literature, as *Ammi majus* L. (Fig.1) and *Clinopodium menthifolium* (Host) Merino subsp. *ascendens* (Jord.) Govaerts (Fig.2). About the principal medical applications, the cited medical species were used mostly as Tonic (e.g. *Polygala vulgaris* L.), as Exciting (e.g. *Origanum vulgare* L.) and as Diuretic (e.g. *Angelica sylvestris* L.). What has been extrapolated till today from the ancient *herbarium* leads to further studies, with regards to the verification of the medical importance species presence no more confirmed from floristic studies of Monte Pisano; this also to investigate the historical evolution of medical plant use in this part of Tuscany. There's no doubt that the *herbarium* "of royal possession" is priceless historical value also for ethnomedical knowledges which enrich ethnobotanical literature of Tuscany and enhance the Monte Pisano Areas of historical and cultural significance.

Fig. 1. Photo from herbarium tables of *Ammis majus* L.

Fig. 2 Photo from herbarium tables of *Clinopodium menthifolium* (Host) Merino subsp. *ascendesns* (Jord.) Govaerts.

1) P.E. Tomei, S. Trimarchi (2022) Un erbario della flora pisana del 1883. Internos. Pisa University Press pp 75-141.
2) B. Pierini, F. Garbari, L. Peruzzi (2009) Inform. Bot. It., 41(2), 147-213.

## 5.7 = Plant location affects *Cistus monspeliensis* anti-inflammatory and antioxidant activity

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*Cistus monspeliensis* L. (Cistaceae) is a lowland shrub commonly known as Montpelier rock-rose (1). It is widely distributed in the Mediterranean basin, in particular in Sardinia, and is dominant in evergreen garrigue vegetation, where grows on both calcareous and siliceous soils. It is reported that diterpenes are the most abundant class of secondary metabolites present in the plant and exhibit anti-inflammatory and antioxidant activity, among others (2). Ethnobotanical studies have shown that *C. monspeliensis* aerial parts and herbal infusions are used in Sardinian plant-based traditional medicine for wound healing and for the treatment of tick bites, respectively (3).

Given that plant secondary metabolites content varies in response to abiotic factors, we have investigated the influence of plant location on *C. monspeliensis* phytochemical profile and on the resulting biological activity. Aerial parts and roots of *C. monspeliensis* were harvested from eight areas of Sardinia; the hydroalcoholic extracts were analyzed by means of $^{1}$H NMR; the anti-inflammatory and anti-oxidant properties were investigated in vitro using a murine macrophage-like cell line (RAW 264.7) and a human neuroblastoma (SHSY-5Y) cell line.

The extracts differed for both the phytochemical profile and biological activity. Labdane and clerodane analogues were the main components identified. Interestingly, the extracts differently modulated the lipopolysaccharide (LPS) and hydrogen peroxide ($H_2O_2$)-induced cytotoxicity, the expression of IL-6 and IL-1β and $H_2O_2$ detoxification.

To better understand the differences between *C. monspeliensis* extracts, the metals content evaluation is also in progress: indeed it can influence the plant biological activity and metabolomic profile.

These data highlight the importance of taking into account environmental factors when considering medicinal plants' biological activity.

1) D. Papaefthimiou, A. Papanikolaou, V. Falara, S. Givanoudi, S. Kostas, A.K. Kanellis (2014) Front Chem, 2.
2) L.F. Salomé-Abarca, M. Mandrone, C. Sanna, F. Poli, C.A.M.J.J. van der Hondel, P.G.L. Klinkhamer, Y.H. Choi, (2020) Phytochemistry, 176.
3) C. Cappadone, M. Mandrone, I. Chiocchio, C. Sanna, E. Malucelli, V. Bassi, G. Picone, F. Poli, (2020) Plants, 9.

## 5.7 = Female and male *Ceratonia siliqua* flowers: phenolic content, antioxidant activity and cytotoxic effects on cancer cells

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The Fabaceae family includes the perennial evergreen *Ceratonia siliqua* L., often known as carob. This tree is indigenous to the Mediterranean area and has been grown for millennia because of its many useful traits and uses. Carob trees are appreciated for their propensity to thrive in arid and semi-arid situations, making them crucial for attempts to conserve soil and plant trees in arid areas. The plant is useful for forestry operations because of its deep roots, which aid in preventing soil erosion. Additionally, carob trees have nitrogen-fixing nodules on their roots that improve the soil's fertility and promote the growth of nearby plants. Small and unnoticeable, *C. siliqua*'s flowers are often greenish-yellow in hue. They grow on the tree's branches in inflorescences, which are organized in thick clusters. Carob trees are dioecious, which means that the male and female flowers are located on distinct plants or present both in some hermaphrodite subjects. While the polyphenol content of carob fruits and seeds is well established, little is known about the presence and composition of polyphenols in carob flowers. According to studies, *C. siliqua* flowers contain a wide variety of polyphenols, including flavonoids, tannins, and phenolic acids (1). In carob blossoms, flavonoids such quercetin, kaempferol, and rutin have been found. These substances are well-known for their anti-oxidant qualities and possible health advantages. The presence of polyphenols in carob flowers raises the possibility that these floral elements have biological and health-promoting properties (2,3). This study aimed to assess the phytochemical profile of two alcoholic extracts from female (FF) and male (MF) flowers of *C. siliqua* by HPLC-DAD and by determining the total polyphenol (TPC) and flavonoid (TFC) content, as well as the antioxidant properties and the potential cytotoxicity on healthy and cancer cell lines. Through HPLC-DAD analysis we discovered that the FF extract has a qualitatively and quantitatively slightly richer profile than the MF extract. While the Folin-Ciocalteu and Aluminum Chloride, methods revealed that the FF and MF extracts show a similar content of polyphenols (FF: 246.00±0.62 mg GAE/g of extract; MF: 248.44±0.53 mg GAE/g of extract) and flavonoids (FF: 19.55±0.09 mg CE/g of extract; MF: 22.19±0.08 mg CE/g of extract). The antioxidant properties of both extracts were then tested using *in vitro* cell-free tests such as the DPPH test ($IC_{50}$: FF 10.38±0.11 μg/mL; MF 9.23±0.84 μg/mL), SOD-like activity assay ($IC_{50}$: FF 231.20±0.34 ng/mL; MF 145.29±0.48 ng/mL) and Catalase-Like activity assay ($IC_{50}$: FF 108.35±0.91 μg/mL; MF 139.85±0.83 μg/mL). Results highlight, for both extracts, a similar antioxidant trend in the DPPH test, differently FF and MF extracts showed more marked activity, respectively, in Catalase-Like and SOD-like activity tests. We also performed MTT tests to evaluate their cytotoxic activity on CaCo-2 human cancer cell line and HFF-1 healthy human cells, finding that both extracts are safe on normal cells and effective on CaCo-2 cancer cells in a dose-dependent manner, with FF being more active than MF (IC50 72h: FF 122.62 ±.1.01 μg/mL; MF 161.10±0.98 μg/mL). Interestingly, the LDH release test enabled us to rule out necrosis as the primary cause of cell death brought on by phytocomplexes. Further research is needed to explore the full spectrum of polyphenols present in carob flowers and their potential bioactive properties.

1) L. Custódio, H. Serra, J.M. Nogueira, S. Gonçalves, A. Romano (2006) J. Chem. Ecol., 32, 929-942.
2) S. Tasneem, B. Liu, B. Li, M.I. Choudhary, W. Wang (2019) Pharmacol. Res., 139, 126-140.
3) A. Alqudah, E.Y. Qnais, M.A. Wedyan, M. Oqal, M. Alqudah, R. AbuDalo, N. Al-Hashimi (2022) Heliyon, 8:e10795.

## 5.7 = *Achillea millefolium* and *Achillea erba-rotta* subsp. *moschata*: investigation of two species traditionally used in northern Italy

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The genus *Achillea* includes more than 100 species distributed throughout the world and characterised by ethnopharmacological importance. In this work, two species were selected for chemical characterization and testing of their potential action in the treatment of skin disorders. *Achillea millefolium* L. is native to Eurasia, North Africa and North America and has been introduced to various other habitats. It is the most popular medicinal plant of the genus *Achillea*, was used in ancient Greece, and owes its name to Achilles, the Greek mythical hero who used this plant to treat war wounds. The distribution range of the other species, *Achillea erba-rotta* subsp. *moschata* (Wulfen) I. Richardson is limited compared to *A. millefolium*. It is in fact an endemic perennial herbaceous species that grows selectively on the siliceous Alps above 1800 m. Both species are traditionally used both as food and for medicinal purposes. Among the therapeutic properties, they are known as antihypertensive, diuretic, digestive, sedative and for the treatment of skin diseases (1,2). *A. millefolium* has been extensively studied, while there is less work on *A. erba-rotta* subsp. *moschata* (2,3). This project aims to compare different samples of the two species from a chemical point of view with particular attention to their potential biological activity to inhibit enzymes involved in skin care (tyrosinase and elastase). The chemical profile of the hydroalcoholic extract of the plant aerial parts showed the presence of polyphenols, including phenolic acids (e.g., chlorogenic acid) and flavonoids (e.g., quercetin, apigenin derivatives), in agreement with literature data (2,3). Although they had similar chemical composition, a statistical analysis (principal component analysis) performed on the basis of the quantification of the main components allowed the two species to be distinguished. The extracts of the two species also showed interesting tyrosinase and elastase inhibitory activity, the percentage of enzyme inhibition being about 10-15% for both enzymes at a concentration of 16 μg/ml. These findings confirm the presence of bioactive compounds in the hydroalcoholic extracts obtained from these species and support the traditional use of these species in the treatment of skin diseases, making them interesting for the cosmetic and health fields.

1) W. Applequist, D.E. Moerman (2011) Econ. Bot., 65, 209–225.
2) S. Vitalini, S. Garzoli, F. Sisto, R. Pezzani, M.P. Argentieri, A. Scarafoni, S. Ciappellano, M. Zorzan, J. Capraro, D. Collazuol, M. Iriti (2022) J. Ethnopharmacol., 298, 115670.
3) M.P. Argentieri, M. Madeo, P. Avato, M. Iriti, S. Vitalini (2021) Z. Naturforsch, 75, 57–64.

## 5.7 = Metabolomics of *Chamaemelum nobile* green extracts by a combined LC-MS and NMR approach

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*Chamaemelum nobile* (L.) All. (syn. *Anthemis nobilis* L. and *Chamomilla nobilis* Godr.) is a perennial herb of the Asteraceae family, with the common name Roman chamomile. Traditionally chamomile is considered to be an antiseptic, antibiotic, disinfectant, bactericidal, fungicidal and vermifuge. It has been used for centuries as anti-inflammatory, antioxidant, mild astringent, mild sedative, antispasmodic, and antibacterial remedy (1). Different classes of bioactive constituents are present in chamomile, including phenolic compounds.
In recent years, there has been a growing interest in developing ecological and environmentally friendly methods for natural products extraction. These methods are known as "green extractions", which allow for obtaining a higher extraction yield using a lower amount of solvents and energy. They also help to reduce the extraction time if compared to conventional extraction methods. In this project, fresh plant material of *C. nobile* was submitted to different extraction protocols like macerations, ultrasound assisted extraction (UAE), and solid-liquid dynamic extraction (SLDE-Naviglio) using EtOH and EtOH/$H_2O$ (50:50, 60:40, 75:25, 100:0) as bio-solvents.
The extracts were analyzed by a combined approach of LCESI/QExactive/MS/MS and NMR. Both LC-MS and NMR data were analyzed by Principal Component Analysis (PCA), to highlight how the extraction method can affect the chemical profile of the extracts.
Chamomile tea has long been used for calmness and sleep disorders. Some authors reported that the sedative effect is due to a flavonoid, apigenin, found in chamomile (2). Moreover, chamomile has been found to possess ingredients that play important roles in Central Nervous System (CNS) diseases such as epilepsy and Alzheimer's and Parkinson's diseases (2). Based on the evidence that in Parkinson's disease, neurons that contain the dark-brown cytoplasmic pigment neuromelanin are particularly susceptible to neurodegeneration (3), the tyrosinase inhibitory activity of the extracts was herein tested by spectrophotometric assay.

Fig. 1a-1b. *Chamaemelum nobile* fresh plant and extracts.

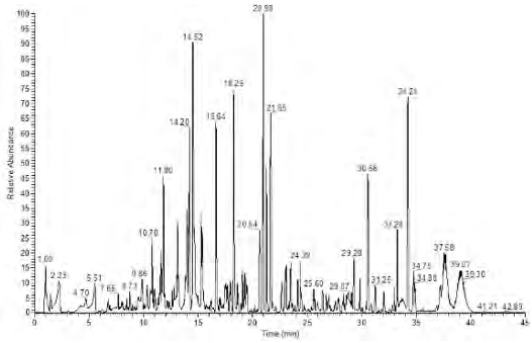

Fig. 2. LCMS profile of EtOH/$H_2O$ 50% extract by maceration.

1) J. Zhao, S. Khan, M. Wang, Y. Vasquez, M. Yang, B. Avula, Y. Wang, C. Avonto, T. Smillie, I. Khan (2014) J Nat Prod., 77, 509-515.
2) S. Amit, P. Naseef, M. Kuruniyan, M. Jain, F. Zakir, G. Aggarwal (2022) Pharmaceuticals, 15, 1284.
3) I. Carballo-Carbajal, A. Laguna, J. Romero-Giménez, T. Cuadros, J. Bové, M. Martinez-Vicente, A. Parent, M. Gonzalez-Sepulveda, N. Peñuelas, A. Torra, R. Rodríguez-Galván, A. Ballabio, T. Hasegawa, A. Bortolozzi, E. Gelpi, M. Vila (2019) Nat Commun., 10, 973.

## 5.7 = Composition and biological activity of essential oil of *Artemisia herba-alba* harvested in Tunisa

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The genus *Artemisia* (Asteraceae) includes about 500 species mainly distributed in the temperate zones of the Northern hemisphere. *Artemisia herba-alba* Asso, also known as desert wormwood, has been used by many cultures since ancient times as an aromatizer for tea and in folk medicine for the treatment of colds, cough, and intestinal disturbances (1). Pharmacological evidence has also attributed to this plant antidiabetic, antihypertensive, antioxidant, antifungal and anthelmintic activities. In recent years, research has focused on its essential oils (EOs), whose composition reveals the presence of numerous chemotypes depending on the geographic origin (2), with different bioactivity. Leaves and branches of *A. herba-alba* grown in Tunisia were cleaned of dust, soil and other residues and dried in the air. The EOs were obtained by hydrodistillation and their composition was determined by GC and GC-MS methods. The antioxidant activity was determined using the 2,2-diphenyl-1-picrylidrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) (ABTS) assays; moreover, cell viability and nitrite quantification were evaluated. The chemical composition shows the presence of camphor, eucalyptol, thujone, camphene, borneol and chrysanthenone as the major components. The composition agrees with the literature where these terpenes are present among the main components (1-2). These compounds have been reported to be related to anti-inflammatory and antioxidant activities (3). The EO showed antioxidant activity in both assays and antinflammatory potential.

1) H. Monsen, F. Ali (2009) Molecules 14, 1585-1594.
2) A. Moufid, M. Eddouks (2012) PJBS 15, 1152-1159.
3) A.E.H. Mohamed, M.A. El-Sayed, M.E. Hegazy, S.E. Helaly, A.M. Esmail, N.S. Mohamed (2010) Rec. Nat. Prod. 4, 1-25.

## 5.7 = Officinal plants and functional foods in the treatment and prevention of cardiovascular diseases: from the ethnobotanical tradition of Italy to the scientific approach

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Ethnobotany, ethnomedicine, biodiversity, phytochemistry, and nutrition are inextricably linked, but suffer from a compartmentalization and lack of communication that must be overcome if progress is to be made. It is by starting with the ancient ethnobotanical tradition, and then flowing into the scientific literature of recent years, that an analysis of medicinal and food plants found in Italy and used in the treatment and prevention of cardiovascular disease can be made.
In this work, we set ourselves the goal of analyzing medicinal plants found in Italy and used in the treatment and prevention of cardiovascular diseases, starting with knowledge derived from the country's ethnobotanical tradition and arriving at the scientific literature produced in recent years. This was to establish scientific validation to the therapeutic efficacy of plants traditionally known in the territory itself. A systematic literature search was conducted, dwelling on publications developed between 2011 to 2022. Botanical databases were also consulted to validate the nomenclature of plant species, verify their phylogenetic characteristics and spatial distribution. Ninety-six plants present in Italy, distributed in 41 families and 84 genera, with a history present in the ethnobotanical cultural heritage, were identified for their role in the treatment and prevention of cardiovascular diseases.
The results showed that officinal and food plants, often typical of the Mediterranean diet, are linked to pharmacological properties that are often expressed in the context of the disorders considered, such as: antihypertensive, hypolipidemic, cardioprotective, cardiotonic, antiarrhythmic, antiplatelet, and antithrombotic. Their high nutritional qualities are emphasized in the literature, such as to give them both a role as functional foods and for possible nutraceutical applications: in fact, all the phytochemical characteristics of the plant species analyzed have been traced and linked, and, to the presence of specific bioactive compounds, the molecular mechanisms upstream of their pharmacological effects have been investigated.

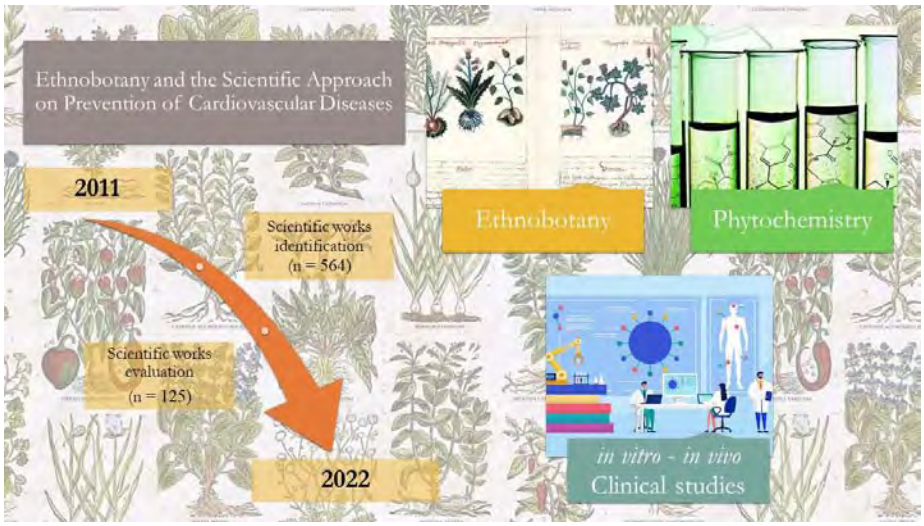


Fig. 1. Research workflow.

## 5.7 = $^{1}$H-NMR analysis and in vitro assay of flavonoids, carotenoids, polyphenols and antioxidant activity of wild roses

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The genus *Rosa* includes about 150 European species used in traditional medicine, in cosmetics and food industry.
This work was based on eight species of wild *Rosa* from the Apennines (*Rosa arvensis* Hudson, *Rosa canina* L., *Rosa cinnamomea* L., *Rosa dumalis* Bechst., *Rosa gallica* L., *Rosa rubrifolia* Vill., *Rosa sempervirens* L., *Rosa tomentosa* Sm.) grown *ex situ* in the botanical garden of Bologna to minimize the morphological and phytochemical differences due to environmental influences.
The aim was to compare the rosehips of these species in term of total flavonoid, polyphenols and carotenoid assay and *in vitro* antioxidant activity test (DPPH). The comparison was performed during the ripening process, from June 2022 to January 2023 (Fig.1) and it was also performed a $^{1}$H NMR-based metabolomic analysis (1) in order to have a more complete picture of the metabolite present in the samples. Significant statistical differences ($p < 0.0001$) were found between the various stages of maturity, characterized by a progressive lowering of total flavonoids, polyphenols and antioxidant power and an increase in total carotenoids. Differences were detected also among the species (Fig.2), identifying *Rosa gallica* as the richest specie in flavonoids, carotenoids and antioxidant power, followed by *Rosa rubrifolia* for its content of flavonoids and antioxidant power. The highest content in polyphenol is showed by *Rosa dumalis* and *Rosa cinnamomea*.
The PCA (Principal Component Analysis) developed on $^{1}$H NMR spectra allowed us to follow the trend of maturation of the rosehip, highlighting that during the ripening process there was a decrease in procyanidins, amino acids and quinic acid and a consequent increase in glucose, citric and formic acid. An intermediate stage was also identified (during September), in which there was a high production of glycine, potentially due to hydric stress. Moreover, the PCA showed different trends of metabolomic variation over time for some species. In particular, *R. gallica* had the highest content of procyanidins also during September when other species were already lowering these compounds (Fig.2).
Compared to the others, *R. rubrifolia* and *R. tomentosa* had a premature ripening, and *R. rubrifolia* showed a high content of proline during diverse stages of its maturation. Considering the role of this amino acid as an osmolyte, its high content could reflect a higher sensitivity of this species to hydric stress. Differently from the other species, in *R. semprevirens* it was possible to detect the presence of procyanidins also in the latter stages of ripening from November to January. Further studies will be performed by LC-MS to evaluate the metabolites which are present at low concentrations.

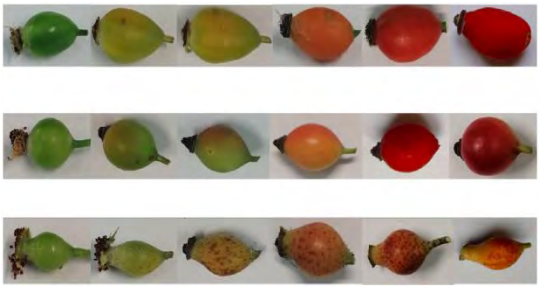

Fig. 1. Color evolution of rose hips in different stages of maturation.

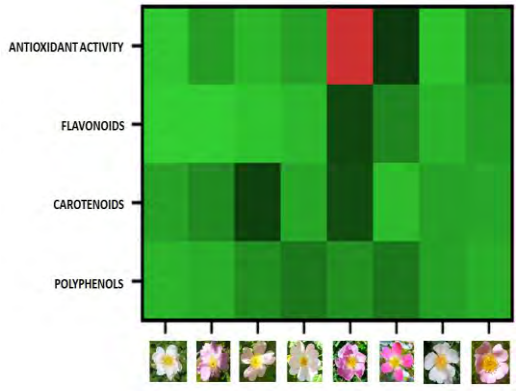


Fig. 2. Heat map of rose hips at maturation.

1) Mandrone et al. (2021) Food Control, 127, 108141

## 5.7 = Chemical characterization and biological evaluation of *Momordica charantia* fruit extracts for their wound healing properties

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An ethnobotanical study conducted in Northern Greece revealed that the fruit of *M. charantia* L. immersed in seed oil is an herbal preparation applied for its wound healing properties by most of the informants in the study area (1). For the study of this traditional preparation, the fruit was separated from the oil and extracted via Ultrasound Assisted Extraction (UAE) using successively solvents of increasing polarity, *c*-hexane, ethyl acetate (EtOAc), methanol (MeOH) and methanol:water 50:50 (MeOH:$H_2O$ 50:50) (Fig. 1). The chemical composition of the remaining oil and the hexanic extract was analyzed using GC-MS (2). Using seed oil as control, the analysis demonstrated that the composition of the preparation oil, hexanic extract, and control oil is almost identical. The EtOAc and the MeOH fruit extracts obtained by the extraction procedures described above, were free from the remaining oil using liquid-liquid extraction, while the methanolic extract (MP) was further fractionated employing Fast Centrifugal Partition Chromatography (FCPC). Structure elucidation of secondary metabolites (mainly triterpene saponins) was facilitated by 1D&2D-NMR experiments.

Moreover, the species was cultivated, and the fruits were lyophilized and extracted via UAE successively using EtOAc, MeOH and MeOH:$H_2O$ 50:50. Then, the methanolic fruit extract obtained by the traditional preparation (MP) was compared to the methanolic fruit extract (M) from the cultivation. The chemical characterization of M and MP extracts was carried out using High-Performance Thin-Layer Chromatography (HPTLC), High-Performance Liquid Chromatography coupled with a Diode Array Detector (HPLC-DAD) and Liquid Chromatography-Mass Spectrometry (LC-MS).

The activity of M and MP extracts on cell viability has been evaluated by the 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, using HaCat human keratinocytes and L929 murine fibroblasts as skin cell experimental model. On HaCat cells, both extracts demonstrated a very low cytotoxic effect at the highest concentration tested, after 24 h, while an interesting proliferative effect was detected for the M extract at the lowest concentration tested (50 µg/mL). On L929 cells, at the endpoint of 48 h, a dose-dependent decrease of cell viability for increasing extract concentrations was observed, with a steeper slope for exposure to MP, revealing a more hypersensitive response. The wound-healing activity of M and MP extracts was evaluated by *in vitro* scratch wound assay on HaCat and L929 monolayers (Fig. 2). Regarding HaCat cells, all tested concentrations (10, 50 and 100 µg/mL) showed a cicatrizing effect. Regarding L929 cells, M and MP extracts at the lowest concentrations tested (10 and 50 µg/mL), induced a wound closure comparable to that induced by the positive control allantoin, followed by a significant decrease of the effect at the higher concentration tested (100 µg/mL), especially for the MP extract.

The phytochemical characterization of *M. charantia* extracts, coupled with their biological evaluation on skin cell lines, prospects for the discovery of new bioactive natural compounds for the treatment of skin diseases.

Fig. 1. *M. charantia* extraction procedures.

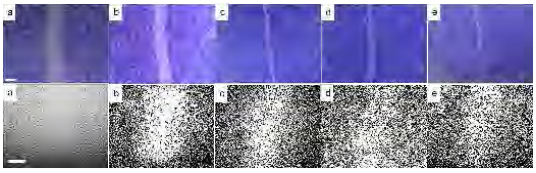

Fig. 2. Scratch wound assays. First line Hacat (24h): a-T0, b-ctrl, c-50 µg/mL M, d-50 µg/mL MP, e-all 50 µg/mL (bar = 500 µm). Second line L929 (48h): a-T0, b-ctrl, c-10 µg/mL M, d-10 µg/mL MP, e-all 50 µg/mL (bar = 300 µm).

1) E.E. Tsioutsiou, P. Giordani, E. Hanlidou, M. Biagi, V. De Feo, L. Cornara (2019) Evid. Based. Complement. Alternat. Med., 2019, 1-22.

2) N. Aligiannis, E. Kalpoutzakis, S. Mitaku, I.B. Chinou, (2001) J. Agric. Food. Chem., 49, 4168-70.

## 5.8 = *Corylus avellana*: a source of diarylheptanoids with α-glucosidase inhibitory activity evaluated by *in vitro* and *in silico* studies

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*Corylus avellana* L. (Betulaceae) (hazelnut tree) is one of the most popular nut trees on a worldwide basis. Hazelnut is a nutritious food with a high content of healthy lipids, used by the confectionary industry and consumed raw (with the skin) or roasted (without the skin) (1).
Our previous investigations focused the attention on the leaves, flowers, shells, green leafy involucres, and kernel of *C. avellana* cultivar "Tonda di Giffoni," leading to the isolation of natural compounds belonging to the class of flavonoids, caffeic acids, and the unusual diarylheptanoids, named giffonins (1).
Type 2 diabetes mellitus is a metabolic disorder in which the body does not produce enough insulin or does not respond to it correctly, developing hyperglycemia (3). One of the strategies for treating this disorder consists in the inhibition of the enzymes that hydrolyze the carbohydrates. One of these is represented by the α-glucosidases which, in the small intestine, metabolize oligosaccharides in single glucose molecules. Therefore, the inhibition of the α-glucosidase enzyme represents an interesting strategy in the treatment of diabetes, since it can retard the uptake of dietary carbohydrates and reduce post-prandial hyperglycemia (2).
With the aim to define the bioactivity profile of giffonins, based on their structural correlation with natural products exerting α-glucosidase inhibitory activity 3), the inhibitory effects of giffonins isolated from *C. avellana* byproducts against α-glucosidase enzyme were evaluated. Herein, molecular docking experiments disclosed the establishment of several key interactions between all the screened diarylheptanoids and the protein counterpart, whose model was built through homology modeling procedure, thus rationalizing the detected inhibitory activities. Specifically, the most active compounds giffonin J, K, and P were able to make both H-bonds and p–p stacking contacts with different residues belonging to the binding site responsible for the catalytic activity of the investigated enzyme. To highlight the occurrence of the bioactive diarylheptanoids in the extracts of *C. avellana* byproducts obtained by eco-friendly extractions, their LC-MS profiles were analyzed. LC-MS analysis showed how giffonin J, K, and P occurred in the ethanol extract of the leaves, while in the extracts of shells and green leafy involucres only giffonin P was evident. Moreover, the quantitative analysis of giffonin J, K, and P in *C. avellana* byproducts was carried out by an analytical approach based on LC–ESI/QTrap/MS, using the Multiple Reaction Monitoring (MRM) experiment. The results of this study prompt us to evaluate *C. avellana* byproducts, especially the leaves, as a prospective source of bioactive diarylheptanoids for the development of functional ingredients for the treatment of hyperglycemia.

1) A. Bottone, A. Cerulli, G. D'Urso, M. Masullo, P. Montoro, A. Napolitano, S. Piacente (2019) Planta Med., 85, 840-855.
2) A. Brown, D. Anderson, K. Racicot, S.J. Pilkenton, E. Apostolidis (2017) Front. Nutr., 4, 56, 1-8.
3) K. Chiba, H. Ichizawa, S. Kawai, T. Nishida (2013) J. Wood Chem. Technol., 33, 44–51.

## 5.8 = Supercritical $CO_2$ extracts rich in palmitoylethanolamide from *Glycine max* waste and *Medicago sativa* aerial parts

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*Glycine max* (L.) Merr. (soy) and *Medicago sativa* L. (alfalfa) (Fabaceae family) have long-standing traditional uses and hold significant importance in the fields of food and health. Soy is renowned for its nutritional properties and versatile applications in food products, while alfalfa is highly regarded as a nutritious forage crop for livestock (1). However, it is worth mentioning that soybean processing generates considerable waste which pose challenges in terms of disposal and environmental impact. Moreover, both soy and alfalfa contain beneficial substances such as *N*-acylethanolamines (NAEs), saponins, polyphenols, and phytosterols, further contributing to their nutritional value and potential health benefits. In particular, NAEs are minor components of plant lipids with anti-inflammatory activity by acting on the endocannabinoid system. The potent biological activity of NAEs has led to the exploration of therapeutic applications, highlighting their anti-inflammatory, antitumoral and antiviral properties. Among these NAEs, palmitoylethanolamide (PEA), is present in significant quantities in both alfalfa and soy (2). PEA is a bioactive lipid, derived from palmitic acid and ethanolamine, that mediates anti-inflammatory, neuroprotective and immunosuppressive activity through the activation of peroxisome proliferator receptor (PPAR α).

The objective of this study was to extract the lipophilic fraction of *G. max* waste and *M. sativa* aerial parts to obtain an extract rich in PEA, considering its reported activities in the literature (3). The environmentally sustainable and clean technology of supercritical fluid extraction was employed for both plant materials, utilizing $CO_2$ under three different experimental conditions including high-pressure extraction (HPE) with 96% ethanol as a co-solvent, HPE without a co-solvent, and low-pressure extraction (LPE) without a co-solvent. This technique avoids the use of toxic organic solvents, ensures high-quality and pure extracts, and prevents degradation of active compounds by extracting the lipophilic fraction of plant matrices. Quantification of PEA was conducted on the three extracts of *M. sativa* and the three extracts of *G. max* by using an external standard to determine the amount present in each sample. Solutions at known concentrations of PEA (concentration range 1- 0.0625 mg/mL) were prepared and injected in triplicate into the UHPLC-ESI-HR-Orbitrap/MS system for constructing a calibration curve. Based on the results, the highest amounts of PEA were found in the alfalfa and soy waste extracts obtained through HPE using 96% ethanol as co-solvent, yielding 65.5 ± 3.1 and 40.5 ± 2.1 mg/100 g of dry extract, respectively. PEA could protect the central nervous system during viral infections, keeping the immune response under control, and exerting a direct action towards viral replication, enhancing pathways and changing the vesicular pH. For this reason, PEA-rich extracts were evaluated against the Zika virus, a + ssRNA neurotropic virus belonging to the Flaviviridae family, both exhibiting a potential antiviral activity against the virus.

1) Y. Li, H. Yang, Z. Li, S. Li, J. Li (2023) Int. J. Mol. Sci., 24, 2620.
2) B. J. Venables, C.A. Waggoner, K.D. Chapman (2005) Phytochemistry, 66, 1913-1918.
3) R. Fonnesu, V.B.S.C. Thunuguntla, G.K. Veeramachaneni, J. S. Bondili, V. La Rocca, C. Filipponi, P.G. Spezia, M. Sidoti, E. Plicanti, P. Quaranta, G. Freer, M. Pistello, M.L. Mathai, M. Lai (2022) Viruses, 14, 1080.

## 5.8 = Development and optimization of a high throughput chlorophyll removal method

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Chlorophylls are green pigments present in photosynthetic organisms such as cyanobacteria, and green plants and algae. Due to their UV/vis absorbance and their natural fluorescent properties, these pigments interfere with analysis and quantification of selected analytes and with biochemical assays that rely on fluorescent or absorbance measurements (1). Moreover, they can easily undergo auto-degradation processes or precipitate in water mediums. Over the years, many different efficient chlorophylls removal methods have been developed, but they are often associated to indirect and involuntary removal of potentially bioactive metabolites and other drawbacks (2). In addition, many of these techniques cannot be applied in parallel to different plants extracts, making them difficult to apply in HTS campaign. The aim of this work is therefore the development of a rapid, economical, easy to use protocol with good performance characteristics and wide applicability to degreen plants extracts. Particularly, herein we report a novel SPE protocol that uses cheap and commercially available cartridges and methanol as solvent of choice. The optimal conditions have been set up exploiting *Coryllus avellana* extracts and next applied to a large set of samples deriving from leaves belonging to different species. All the extracts have been analyzed via HPLC method before and after the SPE treatment and all chromatograms resulted comparable. Moreover, chlorophyll removal has been quantified via a well consolidated UV method (3) demonstrating that our new developed protocol was able to remove more that 85% of both chlorophyll a and b. Finally, all the extracts have been analyzed via both Folin-Chocalteu and DPPH assay demonstrating that our method allowed to maintain unvaried the total phenolic content and the free radical scavenging properties.

1) Seon Beom Kim, Jonathan Bisson, J. Brent Friesen, Guido F. Pauli, Charlotte Simmler (2020) J. Nat. Prod., 83(6), 1846-1858.
2) Rutkowska Ewa; Łozowicka Bożena; Kaczyński Piotr (2018) Food Anal. Methods, 11, 709−724.
3) Robert J. Porra, William A. Thompson, Paul E. Kriedemann (1989) Biochimica et Biophysica Acta (BBA) – Bioenergetics, 975(3), 384-394.

## 5.8 = Green method comparison and optimization for the recovery of anthocyanins from grape pomace

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The exploitation of plant-derived wastes and by-products, and the development of more sustainable methods for the extraction of bioactive compounds still present in these matrices are two out of the goals to be achieved to face the current challenges, such as climate change, biodiversity loss, and increasing environmental pollution.
The wine production chain generates large amount of organic and inorganic wastes. It has been estimated that about five tons of solid waste per hectare per year are produced during cultivation and harvesting (1). Grape pomace is the main solid organic by-product from wine industries deriving from pressing and/or fermentation processes, being mainly composed by seeds and skin, which still contain a wide array of high value-added compounds (2). Among these, anthocyanins are of particular interest, since they can be re-used for different applications, such as for food supplement, pharmaceutical and cosmetic industries, thanks to the well-recognized beneficial effects on human health (3).
The aim of this study was to optimize the anthocyanin recovery from grape pomace by means of green methods. Grape pomace, resulting from the wine production process of *Vitis vinifera* L., cv Sangiovese, was kindly supplied by Podere dell'Angelo winery (Vergiano, Rimini, Italy). Water acidified with 1% citric acid, 50% ethanol acidified with 1% HCl, and a mixture of Natural Deep Eutectic Solvents (choline chloride: citric acid=2:1, with 30% water) were used as green solvents; Microwave-Assisted Extraction (MAE) and Ultrasound-Assisted extraction (UAE) were used as green methods, while the classical solvent mixture consisting of methanol, water and formic acid, and the stirring methods were used as reference protocols.
All the extracts were tested for in vitro antioxidant activity by DPPH assay, and the HPLC-DAD analysis were carried out to determine the targeted anthocyanins concentrations. All the green extracts resulted more enriched in anthocyanins compared to the conventional extract, and MAE turned out to be more efficient than UAE. Hence, MAE was chosen as green technique for the recovery of anthocyanins from grape pomace and the processes were optimized using the Design of Experiment approach (DOE) by means of the MODDE® Pro 13.0.2 software (Sartorius Stedim Biotech, Germany). The extraction conditions were optimized with the application of a central composite face centered design (CCFC), considering three factors: temperature, time, and solid-to-liquid ratio, coding each independent variable at three levels between −1, 0 and +1. Two types of responses, namely the antioxidant activity of the extracts and the concentrations of the targeted anthocyanins, were measured.
Results differed depending on the response and the type of solvent. Overall, when considering antioxidant activity, the optimal extraction temperature is higher than when targeting anthocyanin concentrations. HPLC-DAD analysis revealed that, raising the temperature, the aglycone parts of the anthocyanins became the main components. Precisely, cyanidin and delphinidin were only detected in the extracts obtained at temperature above 90 °C, instead of peonidin-3-O-glucoside, petunidin-3-O-glucoside, oenin and kuromanin which were probably degraded, and they are responsible for the increasing antioxidant activity measured by DPPH assay. In conclusion, results indicate that the choice of the best response is crucial when applying a DOE approach. If the aim is to obtain an extract enriched in specific anthocyanins, the DPPH assay might not be the most appropriate assay, and a targeted chemical analysis is required to precisely characterize the optimized extract.

1) M.P. Zacharof (2017) Waste biomass valorization, 8(4), 1011-1025.
2) C. Beres, G. Costa, I. Cabezudo, N. da Silva-James, A. Teles, A. Cruz, C. Mellinger-Silva, R. Tonon, L. Cabral, S. Freitas (2017) Waste Manage., 68, 581-594.
3) M. Lianza, L. Marincich, F. Antognoni (2022) Antioxidants, 11(11), 2169.

## 6.1 = Upscaling the vegetation shifts along elevation in the GLORIA network peaks using long-term remote sensing time series

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Climate change is causing remarkable impacts on plant species distribution and communities in the cold biomes, with particular concern to arctic and alpine tundra. In alpine ecosystems, the temperature increase is causing an upward shift of species and communities, seeking for the optimum growth conditions. As a prominent effect, a progressive increase of vegetation cover is leading an arctic and alpine greening (1), with far reaching consequences for the species persistence and the overall plant diversity (2,3). Nonetheless, the overall trend is expected to produce different effects along the elevation gradients, but few is known on the processes occurring at this scale. To get new insight into the ecological mechanisms involved in these changes it is important to promote innovative upscaling approaches, able to link field monitoring evidence to remote sensing data. This study aimed at parsing the trends of remote sensing-derived vegetation indices over time in five GLORIA (Global Observation Research Initiative in Alpine Environments) network target regions, located across the Alps and the Apennines in Italy. Some vegetation indices, including NDVI (Normalized Difference Vegetation Index), were calculated for growing seasons (June-September) in the period 1985-2022, using Landsat 5 (1985-2011) and Landsat 8 (2013-2022) multispectral satellite images of each mountain summit. Linear mixed-effects models were used to analyze the relationships between vegetation indices, time and climate variables, derived from CHELSA climate data in the different elevation zones. Over the last 37 years, a linear increase in NDVI was found, but different trends emerged along the elevation gradient, with significant higher increase rates and NDVI values at the treeline, lower alpine and alpine zones when compared to the upper alpine, subnival and nival belts (Fig.1). Moreover, NDVI was significantly affected by temperature at lower altitudes, with a significant interaction with rain precipitations, while climate variables were not determinant at high elevations. These results provided further evidence of the ongoing alpine greening and showed that vegetation at the treeline is responding faster than the other communities to a warmer climate, especially when the amount of water is not limiting. We suggest that future scenarios depicting the fate of alpine plant community should not neglect for the interplay of temperature and precipitation regimes. Our finding open future perspectives on the interpretation of GLORIA field evidence, in a continental upscaling perspective.

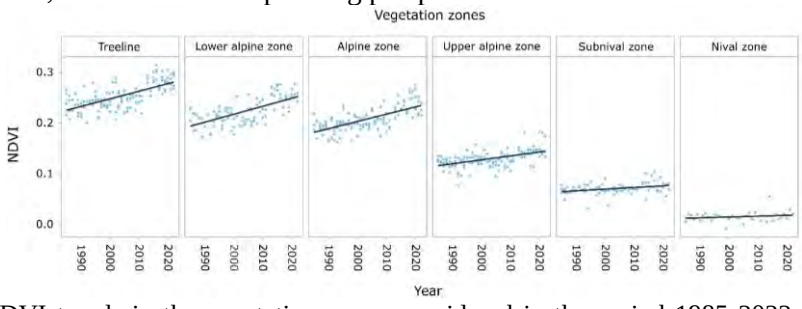


Fig. 1. Contrasting NDVI trends in the vegetation zones considered in the period 1985-2022. The NDVI index was calculated as the average of the growing season (June to September). Effects plots of selected linear mixed model.

1) I.H. Myers-Smith, J.T. Kerby, G.K. Phoenix, J.W. Bjerke, H.E. Epstein, J.J. Assmann et al. (2020) Nat Clim Change, 10, 106–117. doi:10.1038/s41558-019-0688-1

2) A.D. Bjorkman, I.H. Myers-Smith, S.C. Elmendorf, S. Normand, N. Rüger, P.S.A. Beck et al. (2018) Nature, 562, 57–62. doi:10.1038/s41586-018-0563-7

3) F. Boscutti, V. Casolo, P. Beraldo, E. Braidot, M. Zancani, C. Rixen (2018) PLOS ONE, 13, e0196653. doi:10.1371/journal.pone.0196653

**6.1 = Street trees of Rome and their relationship with the changes of urban climatic parameters**

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Natural ecosystems nowadays are being damaged by the increasing urbanization and environmental quality of cities. It is now known that cities significantly impact climate change as they are the main ones responsible for greenhouse gas emissions producing up to 70% of emissions caused by fossil fuel consumption. At the same time, cities are affected by the effects of climate change; therefore, the tree sector and the climate mitigation that it guarantees allow cities to play a vital role in addressing climate change [1]. The purpose of this work is to understand what relationship exists between local climatic conditions and Rome street trees chorotypes (as indicators of adaptation to different conditions) found in different municipalities. We elaborated on both meteorological data and ecological data. Considering seven meteorological stations (within the GRA ringing road) we processed the data between 2008 and 2013 and from 2019 to 2022. Considering Rivas-Martinez [2] thermicity and ombrothermicity bioclimatic indices we could define the bioclimatic context of the different areas identified by the stations. For ecological data, we performed an analysis of species chorotype based on a previous dataset [3] Moreover we analysed their exoticness, discriminating between archeophyte and neophyte, and their invasiveness. Our data show a heat island effect in the in the center stations, belonging to the upper Thermomediterranean thermotype. These data bring to light not only a certain ombrothermal spatial and temporal variability. However, also a transitional situation toward a less xeric bioclimatic region in the western area, closer to the coast and thus more subject to maritime influence, toward the eastern area. In all *municipia* the alien species exceed the 60% and regarding alien species most species are neophytes compared to archaeophytes in all the city. Among the exotic species, naturalized species prevail over casual species. Indeed, for the most represented chorotypes, we find the most significant presence of E-Asiat (24.35%), N-Amer (19.09 %). Only the 15.14 % and 11.01 % are respectively Eurimedit and Steno-Medit, similar to Silv-cult (14.03 %). Regarding the distribution in the various *municipia*, we can see that the E-Asiat chorotype dominates over almost all *municipia* except for municipia IV and VI. Here the Eurimedit chorotype dominates where the ombrotype changed from lower sub-humid to the upper dry. We believe that in the selection of street trees should be favoured autochthonous species more adapted to warm climates in more critical bioclimatic zones highlighted than others. Therefore, this preliminary analysis could lead to greater attention in the planting and design of roadside tree plantings.

1) G. Carter, Jeremy (2011) Current Opinion in Environmental Sustainability, 3(3), 193-198.
2) Salvador Rivas-Martínez, Salvador Rivas-Saenz, Angel Penas (2002) Worldwide bioclimatic classification system. Kerkwerve, The Netherlands: Backhuys Pub.
3) D'Amato et al. (2023) Distribution pattern of urban street trees in Rome (Italy): A multifactorial evaluation, in press.

## 6.1 = Effects of drought timing and warming on primary production, growth and senescence of *Carex curvula* in an experimental alpine grassland

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Future climate scenarios modeled for the Alps predict an increase in temperature and in frequency and intensity of drought events (1) that could affect biodiversity and ecosystem functioning of several ecosystems, such as grasslands. Drought could be a threat to these ecosystems due, for example, to its effects on growth and mortality (2). In general, plant responses have been found to vary according to drought intensity, frequency and timing at which drought occurs (3). Many grasslands are experiencing drought stress globally, but alpine grasslands are by far the less studied in comparison with grasslands from other climates. *Carex curvula* is the dominant species of the most widespread acidophilous grasslands in the Alps. Therefore, understanding the effects of drought events on this species is of paramount important in terms of biodiversity conservation and management of high alpine pastures.
To contribute to this important task we set up a mesocosm experiment to test the effects of drought timing on the growth of *C. curvula*, focusing on (i) the aboveground net primary production (ANPP), (ii) the growth of photosynthetic tissue and (iii) senescence (i.e. the length of the yellow tip of the leaf).
The mesocosm has been prepared using monoliths collected from the field research site, located in the Gavia Valley (Stelvio National Park); then they have been transferred into pots and relocated at the University of Parma's Campus, where they have been maintained in an incubator for 5 months at 0 °C. At the beginning of February 2020, when the air temperature of Parma reached the growing season's temperature at Gavia Pass, the pots were saturated with distilled water to simulate snowmelt. Afterwards, to simulate drought event, they have been positioned below a transparent shelter built with polythene foil. We induce five different treatments: (a) Control, continues watering for 2 months; (b) early-season drought, no watering for 1 month after snowmelt, then watering for the following 1 month; (c) mid-season drought, watering for 1 month and then 1 month of no watering; (d) full season drought, no watering throughout the whole experimental period and (e) full season drought + warming, no watering throughout the whole experimental period coupled with a passive warming treatment.
Our result showed that all drought treatments reduced ANPP (measured at the end of the experiment) in comparison with the control. Leaf growth showed no difference between treatments during the early-season drought period, while the mid-season treatment significantly reduced the length of green tissue during the second month of the experiment. The length of green tissue produced from the beginning until the end of the experiment showed a significant decrease, compared to control, in both early- and mid-season drought, but, as expected, not as marked as the full drought treatment. In *C. curvula* the leaf tip become progressively yellow from the tip towards the base during the second part of the growing season (i.e. senescence). Senescence was not affected by the early-season treatment, but the length of the yellow tip of the leaves was greater in the other treatments in comparison with the control. These results point out that a drought event during the first month of growing season does not affect senescence, but a mid-season drought may accelerate senescence during the second month of the growing season.
Our findings suggest that ANPP is strongly influenced by drought events and that leaves growth and senescence can be modulated by drought timing. These results help to understand the possible responses of *C. curvula* to drought events occurring in different period of the growing season and their effects on ANPP of one of the most widespread grassland in the alpine region. Its response implies an impact on different ecosystem function, e.g., carbon uptake, alteration of the soil organic matter input from litter, ecosystem respiration or biotic interaction (as competition which may leads to community shift); the modification of this processes is very important inasmuch could contribute to intensify climate change by the alteration of carbon cycle.

1) A. Gobiet et al. (2014) Science of the Total Environment, 493, 1138-1151.
2) B. Muller et al. (2011) Journal of Experimental Botany, 62(6), 1715-1729.
3) J. Seifarth et al. (2021) Ecosphere, 12(7), e03620.

## 6.1 = Tree canopy cover vs herbaceous layer – Climate change mitigation of Urban Parks

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In Urban areas, the vegetation acts as a "natural conditioner" reducing the thermal excesses that characterize the Urban Heat Island (UHI) effect by decreasing the air temperature up to 6 degrees in the Mediterranean area. The consequence is a lower energy demand for building cooling systems, which has an indirect positive impact on air quality, greenhouse gas emissions and global warming (1).
Also, replacing portions of urban surfaces with green areas causes a change in the albedo. The variation in the Earth's ability to reflect the incident solar radiation involves a modification in the amount of heat that contributes to the atmosphere's energy balance (as well as the evapotranspiration cooling effect), resulting in a local variation of the air temperature (2) that can be translated in $CO_2$ compensated.
Green Urban infrastructure with high albedo can, therefore, contribute to reducing the effects of the UHI. As part of the LIFE CLIVUT project, multiple analyses on how urban green infrastructure can reduce air temperature were carried out. The main objective of the LIFE CLIVUT project is to test a participatory methodology to collect data on trees and evaluate them in 4 Mediterranean cities (Thessaloniki in Greece, Cascais in Portugal, Perugia and Bologna in Italy) (3). The results obtained during the Project highlighted how urban trees can reduce the UHI through evapotranspiration and modifying the surface albedo.
In addition, in large urban parks, much of the surface area is constituted by lawn, which also contributes to providing ecosystem services. In this case study, the data collected during the census of the Chico Mendez Park of Perugia, Italy, in combination with the analysis of the satellite images through the GreenPix software developed by Research and Technology, Food and Agriculture Institute (IRTA) in Spain allowed:
1) to quantify the percentages of the different surfaces in Chico Mendez Park in Perugia;
2) to estimate the $CO_2$ storage and the potential $CO_2$ compensated through evapotranspiration by trees and herbaceous vegetation of the park;
3) to estimate the $CO_2$ compensated by the relative increase in albedo value among the different typologies of surfaces identified (tree canopy cover, green lawn, dry lawn, white pathways and asphalt).
Results evidenced that depending on the species, the decreased albedo respect to the reference surface can lead to a negative effect causing a warming of the surface, especially if compared to white gravel pathways. However, the potential $CO_2$ compensation by evapotranspiration of the vegetal species must be considered.


Fig. 1. Chico Mendez Park, Perugia.

Fig. 2. Examples of Chico Mendez Park spatial analysis with GreenPix software.

1) Limor Shashua-Bar, Oded Potchter et al. (2010) International Journal of Climatology, 30(1), 44–57. doi:10.1002/joc.1869
2) Dominique Carrer et al. (2018) Environmental Research Letters, 13(4). doi:10.1088/1748-9326/aab650.
3) Fabio Orlandi, Marco Fornaciari et al. (2022) IForest - Biogeosciences and Forestry, 15(2), 133–40. doi:10.3832/ifor3908-015.

## 6.1 = Divergent responses of mountain forests and grasslands to heat and drought events

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Both natural and managed ecosystems contribute to mitigating climate change through the process of $CO_2$ sequestration from the atmosphere. However, projections of future global climate indicate that extreme weather events will become more frequent and more intense in the coming years. Since heat waves and droughts can alter ecosystem functioning and increase the vulnerability of carbon sinks, this trend represents a potential risk for their contribution to reaching global climate change mitigation goals. Therefore, further efforts are needed to assess the resistance and resilience of different ecosystems and land uses to climate change. In this context, alpine mountain ecosystems face a double challenge: on the one hand, warming in the Alps is occurring twice as fast as in other regions of the planet and drought events are increasingly frequent, on the other hand, socio-economic changes have led to partial land abandonment, with effects on the composition and distribution of plant species and communities.
The main objective of this study is to analyze the impacts of extreme heat and drought events on the functioning of two different ecosystems in the Alps: a European larch forest (*Larix decidua* Mill.) and an abandoned subalpine pasture dominated by *Nardus stricta*, both located in the Aosta Valley region (Italy) at about 2100 m asl and thus experiencing the same climatic conditions. The eddy covariance method was used to measure carbon and water fluxes between the ecosystems and the atmosphere. Radiometric vegetation indices (e.g., NDVI), and field observations related to plant phenology were used to explain the role of timing in determining the carbon and water fluxes impacts. Finally, functional traits and of plant species and quantitative wood anatomy were used to interpret the divergent ecosystem responses from an adaptive perspective.
Results showed that the different heat and drought events observed during the ten-year study period (2012-2022) had variable impacts on ecosystem functioning, depending on the ecosystem type, the timing of the extreme event in relation to the vegetation phenology, the presence/absence of the snowpack. In general, more severe impacts were observed for the grassland compared to the forest. The observed contrasting responses will be discussed by exploring the linkage between the functioning of the whole ecosystem and the adaptive strategies of individual plant species.

## 6.1 = Effects of wood-derived biochar on a European beech (*Fagus sylvatica*) forest

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Forests are crucial ecosystems for controlling atmospheric $CO_2$ levels. However, their C reservoirs, which are mainly found in the soil, are under severe threat from both ongoing climate change and deforestation. The application of specific soil C sequestration strategies may, however, partially counteract these losses. One such strategy could be the pyrolysis of wood and the application of its by-product – i.e. the biochar – to the soils where it was originally harvested. Due to its high C content (>60%) arranged in an aromatic structure, biochar has a high environmental stability. Therefore, the conversion of wood into biochar and its application to forest soils can be considered a viable strategy for increasing both atmospheric C sequestration and forest soil C stocks.
Because biochar is essentially a product that does not occur naturally in the forest, its effects on both forest regeneration (germination and growth of dominant tree species) and litter decomposition (one of the largest forest C pools in managed forests) must be carefully assessed before any application. Although biochar applications appear to have a generally positive effect on forest biomass (1), negative or negligible effects have also been reported. Additionally, although it appears that biochar applications have a positive effect on soil respiration (2), little is known about the effect of biochar applications on the decomposition of low- and high-quality litter within the same ecosystem.
European beech forests (*Fagus sylvatica* L.) represent about 10% of Italian forests and are the dominant ecosystem above 1000 m in the northern Apennines. This makes them an important economic/energetic resource. There are no studies that demonstrate the effect of biochar applications on European beech forest ecosystems. Therefore, the aim of this study was to define the effect of hypothetical biochar applications on both the regeneration of beech forests and the litter decomposition.
To test the effect of biochar application on beech forest regeneration, *F. sylvatica* seeds from the Tuscan-Emilian Apennines were germinated under greenhouse conditions using European beech forest soils mixed with 0% (control; soil only), 10% and 20% (v/v) biochar produced from local hardwoods as substrate. At the end of the germination period (four months), seedlings of similar height were selected to continue the experiment on the effects of biochar on plant growth. Plants were then grown under controlled conditions for five months, with height, number of leaves, length of the most developed leaf and photosynthetic parameters (chlorophyll content and photosynthetic efficiency of the photosystem II) recorded each month. Results showed no effect of biochar on either seed germination or seedling growth. However, a stimulating effect of the applications on chlorophyll content (+9%; $p<0.05$) was observed.
To test the effect of biochar application on early litter decomposition, standard litter of high (green tea bags; surrogate leaves) and low (rooibos teabags; surrogate twins) quality was buried within the above-mentioned forest soils mixed with 0%, 10%, 20% and 100% biochar following Keuskamp et al.'s (3) protocol; This experiment was carried out under field conditions. The decomposition of high-quality litter, as assessed by % mass loss relative to the control, was not affected by any of the biochar application. The decomposition of the low-quality litter was instead reduced by increasing the percentage of biochar applied, but this was only significant when biochar application was 20% and 100% ($p<0.001$). In fact, the decomposition of the low-quality litter was reduced by ca. 4% after the amendment with 20% biochar and by ca. 9% after the amendment with biochar alone.
Overall, the results of this study highlight the lack of negative effects of biochar applications on both forest regeneration and litter decomposition in the investigated European beech forest. In particular, biochar applications appear to be effective in reducing the decomposition of low-quality litter. In conclusion, biochar applications appear to be an efficient strategy for increasing C stocks in Apennines forest soils.

1) S.C. Thomas, N. Gale (2015) New Forests, 46(5-6), 931-946.
2) G. Zhou, X. Zhou, T. Zhang, Z. Du, Y. He, X. Wang, ... C. Xu (2017) Forest Ecology and Management, 405, 339-349.
3) J.A. Keuskamp, B.J. Dingemans, T. Lehtinen, J.M. Sarneel, M.M. Hefting (2013) Methods in Ecology and Evolution, 4(11), 1070-1075.

## 6.2 = Mediterranean green roof matters

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Green infrastructures (GIs) are a pivotal tool for environmental and climate change mitigation, enhancing habitat connectivity and ecosystem services (1). This is particularly relevant within urban contexts, where human activities and grey infrastructures have completely altered natural ecological dynamics (2). Fragmentation, complexity and biological homogenization can be counteracted through reasoned green management strategies such as green roofs (GRs). However, while GRs are widely spread and subjected to specific regulations in the U.S. and in central northern Europe, they are still infrequent in the Mediterranean Area. In addition, little is known about plant communities that grow on Mediterranean GRs, facing limited soil and water availability and wide temperature fluctuations (3). Our study focuses on the spontaneous vegetation of two adjacent Mediterranean GRs in the city of Genoa (NW Italy) to characterize plant communities, their relationship with the growing medium properties and their ecological dynamics. The study site consisted of an extensive (20 cm of growing mix + 15 cm of drainage layer), and an intensive (35 cm clayey ground + 8 cm drainage layer) GRs, covered by spontaneous plant communities. Phytosociological relevées were carried out on both GRs in June and September 2022 (t0 and t1, respectively). After t0, the vegetation was completely removed from 28 plots of 1 $m^2$, evenly placed on the two GRs. After t1, the vegetation was again removed and fresh biomass was weighted. This procedure allows comparing plant communities in different plant succession (well-established *vs* colonizing) in relation to different growing media (extensive *vs* intensive). To this aim, species richness and biodiversity index were computed, then an ANOVA was performed to test the difference in biodiversity index variation between GRs. Differences in species composition were inspected both qualitatively (t0) and through a Non-Metric Multidimensional Scaling (NDMS) (t1). Species turnover indices between t0 and t1 were computed and fresh biomass weight values were compared between the GRs with a Kruskal-Wallis test. Last, the presence and abundance of invasive alien species was recorded. Species richness was higher on the extensive GR, with around 10 species more than the intensive GR, both at t1 and at t0. Biodiversity index showed higher values in the extensive GRs at t1, while the ANOVA did not highlight significant difference between GRs at t1. However, marked differences in species composition between GRs were evident both at t1 through the NMDS and at t0 through the qualitative assessment. Turnover values were higher in the intensive GR, while total fresh biomass was significantly higher in the extensive GR. Among the sampled species, *Portulaca oleracea* L. and *Digitaria ischaemum* (Schreb.) Muhl. were the main contributors to fresh biomass in both GRs at t1. While no alien species was present at t0 in any of the GRs, 4 of them were sampled on the extensive and 2 on the intensive GR at t1. Our findings extend the relevance of the growing media for plant species composition and colonization to Mediterranean GRs and highlight how these GIs display vegetation dynamics similar to those of natural ecosystems, with more generalist species during the colonizing phase and more specialized species in the stable phase. The removal of vegetation after t0 promoted the spread of invasive species. Vice versa, during the stable phase, invasive species were absent, probably counteracted by well adapted native species. Since the management of urban green spaces acts as disturbance for plant communities, our results suggest that strong management regimes do not favour ecological balances, promoting alien species colonization. From a planning perspective, we provide a set of local plant species adapted to the demanding conditions of GRs that can be employed to further promote the spreading of these systems also in the Mediterranean area. In the end, these species play a relevant role in delivering ecological benefits to all organisms, including humans, promoting multitrophic interactions and improving the local biodiversity. Consequently, Mediterranean GRs can contribute to the achievement of key EU's policy objectives (e.g., 2030 Biodiversity Strategy goals) and the UN Sustainable Development Goals (SDGs).

1) European commission, (2019) Report from the commission to the European Parliament, the Council, the European economic and social Committee and the Committee of the Regions. Review of progress on implementation of the EU green infrastructure strategy. Brussels, 24.5.2019.
2) C. Catalano, S. Brenneisen, N. Baumann, R. Guarino (2016) RETICULA, 12(12), 1-10.
3) C. Catalano, R. Guarino, S. Brenneisen (2013) A plant sociological approach for extensive green roofs in Mediterranean areas. 10.13140/RG.2.2.21245.49126.

## 6.2 = Impact of active coppice management on microclimate and understorey vegetation in a Mediterranean oak forest

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Plant diversity of the forest understorey contributes to forest functionality and provisioning of ecosystem services. In recent years, however, understorey thermophilization processes caused by global warming have been detected especially in temperate regions with warm macroclimate, such as the Mediterranean one. Coppice-with-standards is still one of the most common types of management of broadleaved and evergreen forests in this region, aimed at the production of renewable energy, especially firewood. Trees are cut at regular time intervals, thus leading to cycles of vegetative regeneration. However, the strong modification of forest structure caused by coppicing could mine the capacity of forest canopy to buffer temperature extremes and to offer microclimatic refuges for the herb communities. Hence, it is crucial to assess the sustainability of this traditional management form under the current climatic stressors. We contributed to this topic by analyzing shifts in temperature buffering capacity, understorey diversity (taxonomic, phylogenetic, functional), and productivity in an ancient forest of central Italy with *Quercus cerris* and *Q. petraea* (Bosco ai Frati, Tuscany). Here coppice-with-standards and high forest are next to each other and under homogeneous site conditions since long time. To this purpose, in 2021 we installed air and soil temperature dataloggers in three high forest sites and three coppice-with-standards sites. Following a nested sampling design, forest structural variables light availability (photosynthetic active radiation) and soil pH were determined before surveying understory vegetation in four 5 x 5 m randomly selected quadrats in each of the six sites (24 quadrats). Understory aboveground productivity (dry weight of herbaceous and woody biomass) was determined in 48 0.5 x 0.5 m plots (two per quadrat). Moreover, five functional traits associated with the acquisition and conservation of resources (vegetative traits) and reproductive efficiency were collected from the TRY database: Specific Leaf Area, Leaf Dry Matter Content, plant vegetative height, plant reproductive height, and seed mass. Regarding microclimate, we confirmed the strong effect of forests in limiting maximum temperatures. The mean offset values between forest and reference open areas in daily maximum temperatures were significantly larger in the high forest than in the coppice stands during all seasons. Our results supported that coppice management promotes understorey species richness, although this is due to the presence of mostly generalist and light-demanding species with strong colonization capacity. Interestingly, coppicing led to a clustering in phylogenetic structure (measured with Mean Nearest Taxon Distance) and differed significantly from high forest in functional diversity for Leaf Dry Matter Content, highlighting the presence of ongoing acclimation processes in the understorey. Coppicing also influenced the diversity-productivity relationship. In the light of these results, we emphasize the need to take into account different facets of plant diversity, to reach a more holistic understanding of the effects of coppicing on deciduous oak woodlands of the Mediterranean region, on especially plant diversity and temperature buffering capacity of the forest canopy.

## 6.2 = *Ferula communis* and *Cynara cardunculus* communities as inspiring landscapes of Dyonisian cults

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*Ferula communis* L. and *Cynara cardunculus* L. are two steno-Mediterranean typical elements of arid grasslands, that are found as imposing spontaneous populations between Tarquinia and Tolfa Hills (NW of Rome), whose natural importance is enhanced by their cultural value. In fact, these two species are part of xerophilous plant communities (1) that, besides being very interesting from a floristic point of view, were a source of inspiration for Dionysian cults. *F. communis* had a close connection with the Greek God Dionysus, as its hollow stem was used in Dionysian celebrations both as a thyrsus (ritual stick) and to make masks worn in the honour of the God, but also for the preparation of perfumes and ointments used during the rites (2,3). Such thyrsus had in the apex a conical structure, which can be interpreted in some case as the immature inflorescences of *C. cardunculus,* but also as female cones of *Pinus pinea* L. or by arrangements of *Hedera helix* L. plants. Such plants form natural or artificial communities, that inspired the ancient people for rituals and religious purposes. For this reason, in order to investigate the ecological framework, cultural and historical values, and management over time, of the communities of *F. communis* and *C. cardunculus* as priority habitats, we carried out vegetation surveys in the Tolfa hills, and conducted statistical analyses (Cluster analysis, NMDS) to compare these formations with those of other areas in Italy: for *F. communis* Sicily and Marche, for *C. cardunculus* Molise and Calabria (Fig. 1-2). Results revealed that these formations differ from such similar coenoses and can be attributed to different types. From this first comparative analysis, it was possible to take a step forward towards the knowledge of these communities that were as important in the past as they are now, in which cultural, historical and vegetation characteristics have worked in synergy leading to the development of human civilizations and plant communities, revealing their close connection, with the future aim of being able to further manage and conserve these environments of high cultural and vegetation value.

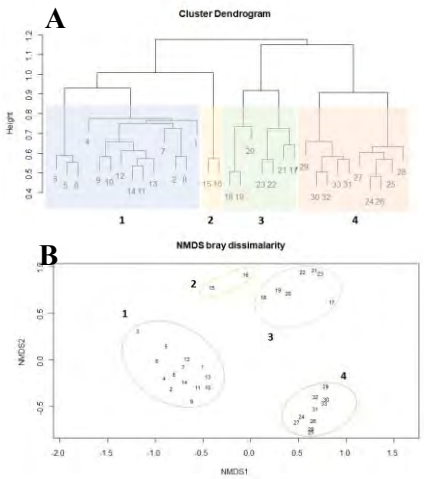


Fig. 1. Cluster (A) and NMDS analysis (B) between *Ferula communis* L. communities: 1) *Ferula communis* L. community in Tarquinia-Tolfa Hills; 2) *Asphodelo ramosi-Feruletum communis* (Biondi et al. 2016); 3) *Asphodelino luteae-Feruletum communis* (Biondi et al. 2016); 4) *Carlino siculae-Feruletum communis* Gianguzzi, Ilardi & Raimondo 1993.

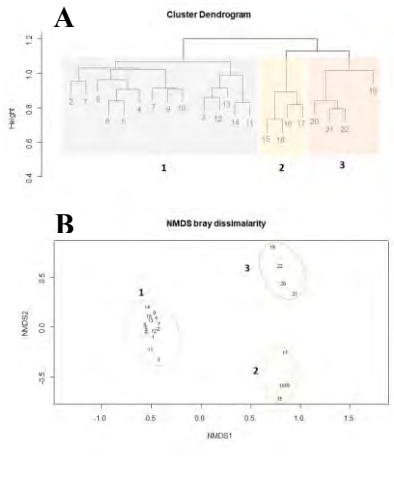


Fig. 2. Cluster (A) and NMDS analysis (B) between *Cynara cardunculus* L. communities: 1) *Cynara cardunculus* L. community in Tarquinia-Tolfa Hills; 2) *Hedysarum coronarium-Cynara cardunculus* community (Bernardo et al. 1991); 3) *Cynaro-Elytrigietum* community (Lucchese and Fanelli 2003).

1) E. Biondi, S. Pesaresi, D. Galdenzi, R. Gasparri, N. Biscotti, G. Del Viscio, S. Casavecchia (2016) Plant Sociol, 53, 3-18.
2) R. Kandeler, W. R. Ullrich (2009) Journal of Experimental Botany, 60(3), 715–717.
3) H. Koerper, A. L. Kolls (1999) Economic botany, 133-143.

## 6.3 = The study of functional morpho-anatomical traits in *Solanum lycopersicum* 'Micro-Tom' in closed ecological systems

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Functional traits are species' morphological and physiological attributes influencing growth, reproduction, or survival of a plant. They are widely used in ecology to have information about communities responses to disturbances and their recovery[1].
Recently, additional set of traits defined 'anatomics', including several plant anatomical characteristics, has been recognized to be an efficient "signal" to study plant acclimation to environmental changes[2,3]. Studying the possible correlations among functional traits and environment assumes particular importance in Closed Ecological Systems (CLESs), that do not exchange matter outside the system itself.
Among CES, Bio-regenerative Life Support Systems (BLSSs) are essential to guarantee the possible permanence of humans in space. In a BLSSs plants, especially crops, play a leading role not only in feeding the astronauts, but also as regenerators of resources, through photosynthetic oxygen production and water purification by transpiration. However, space factors such as altered gravity and ionizing radiation, may represent a significant hazard for plants, since they can determine important alterations of morpho-anatomical, eco-physiological and biochemical traits depending on plant species, cultivar, and development stage.
In this study we performed a ground-based experiment to analyze the effect of X-rays delivered at different doses (0.3, 1,10, 20, and 30 Gy) on *Solanum lycopersicum* cv. 'microtom', a candidate species for space cultivation. The irradiation was performed at the stage of germinated seeds and, after the irradiation, plants were cultivated in a growth chamber under controlled conditions up to fruit ripening (Fig 1).
At harvest, morpho-biometric characteristic (e.g., stem elongation, fresh and dry biomass) as well as leaf functional traits (e.g., specific leaf area, leaf dry matter content, relative water content) were determined together with leaf functional anatomical traits (e.g., lamina thickness, localization of phenolics, stomatal frequency), quantified through light and epi-fluorescence microscopy and digital image analysis (Fig. 2-3). The correlations between all functional traits were considered to assess plant responses to the different doses of ionizing radiation.
Our data indicate that the observed responses strongly depend on the dose. Moreover the change of a specific functional trait linked to a particular dose is important to understand the mechanisms of microtom radio resistance and test the limits of cultivation of this crop in space-simulated conditions. These findings should be considered when studying plant adaptation in CESs and can also have possible spin-off in controlled environment cultivation.


Fig. 1. The irradiation procedure.


Fig. 2. Leaf functional traits.

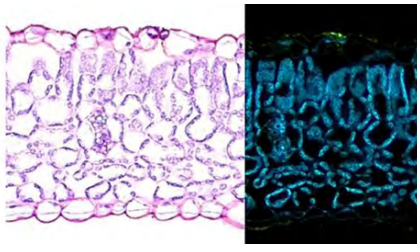
Fig. 3. Light and epi-fluorescence microscopy.

1) P.M. Brousseau, D. Gravel, I.T. Handa (2018) Journal of Animal Ecology, 87(5), 1209-1220.
2) C.F. Strock, H.M. Schneider, J.P. Lynch (2022) Trends in Plant Science.
3) C. Amitrano, A. Junker, N. D'Agostino, S. De Pascale, V. De Micco (2022) Planta, 256(4), 68.

## 6.3 = Why do flower become larger and leaf smaller along elevation gradients? A case study in the Apennines (Italy)

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Variation in the size of plant leaves and flowers is influenced by environmental factors such as soil fertility, temperature, and water availability. Previous research conducted in the Alps has indicated a general decrease in plant size along elevation gradients (1). In this study conducted in the south-central Apennines, our objective was to examine the changes in leaf and flower size of herbaceous plants along elevation gradients. We collected samples from six mountain ranges, namely Majella, Gran Sasso, Laga, Picentini, Lattari, and Alburni-Cervati. A total of 1121 individuals from 130 different species were sampled. Data collection involved ten transects spaced at intervals of 150 meters, spanning from sea level to an elevation of 2912 meters above sea level. Four traits were measured for each individual: corolla diameter, leaf length, leaf width, and leaf area. The results demonstrated a consistent decrease in all leaf-related traits with increasing elevation, with a significant drop observed at 2000 meters above sea level. In contrast, corolla diameter exhibited distinct patterns along the elevation gradient, depending on the number of flowers associated with each species. Species with few flowers or a single flower displayed an increase in corolla diameter with altitude, while species with numerous flowers exhibited a decline in corolla size as elevation increased. These findings highlight the remarkable adaptability of high-altitude species, as evidenced by their ability to alter allometric characteristics in response to climatic conditions. Studying the variations in morphological features with elevation over time could provide valuable insights into the effects of climate change on species that are particularly vulnerable at high altitudes.

1) C. Körner, C. Kèorner (1999) Alpine plant life: functional plant ecology of high mountain ecosystems. Springer Verlag, 1st edition.

## 6.2 = Nectar chemical composition in the tribe Lithospermeae (Boraginaceae) and its possible effect on specialized nectar yeasts

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It is recognized that phylogenetically distinct nectariferous species visited by the same pollinator guild may display a convergent chemistry of their floral nectar revealing a pollinator-driven selection of nectar (1). Cases of divergence in nectar chemistry within a restricted phylogenetic context are of particular interest since they may reveal selection pressures associated to pollinators. The diversity of pollinators also affects the nectar microbiota which in turn may shape the chemical composition of nectar for both primary and secondary metabolites. We analyzed the nectar of more than twenty Lithospermeae species revealing a heterogeneous profile of sugars, amino acids, and secondary metabolites, such as biogenic amines, that act as neurotransmitters in insects.
We found biogenic amines in the nectar of species exhibiting sucrose-dominant nectar chemistry such as *Echium* sp. pl., *Cerinthe major* and *Onosma* sp. pl., while they are almost absent in the nectar of species with hexose-dominant nectar profile, such as *Aegonychon purpurocaeruleum*, although these three species are visited by very similar species of insects, like long-tongued bees. Biogenic Amines in nectar can be the result of endogenous production or be attributed to the metabolic activity of microorganisms such as specialized yeasts that inhabit the nectar (2). To test if the nectar sugar profile affects the ability of yeasts to change the nectar chemistry, four species of yeasts were grown in artificial nectar with three sugar profile: sucrose dominant, sucrose rich and hexose dominant. The artificial nectars were replicated and the yeasts of three strains for each species were inoculated to test their effect on the chemistry of the nectar.

1) S. Abrahamczyk, M. Kessler, D. Hanley, D.N. Karger, M.P.J. Müller, A.C. Knauer, F. Keller, M. Schwerdtfeger, A.M. Humphreys (2017) Journal of Evolutionary Biology, 30, 112-127. DOI: 10.1111/jeb.12991
2) M. Barberis, D. Calabrese, M. Galloni, M. Nepi (2023) Plants, 25,12(3), 550. DOI: 10.3390/plants12030550

## 6.3 = Stomatal patterning in 13 plant species and their relationship with mesophyll conductance

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Understanding the impacts of rising $CO_2$ on plant anatomy and physiology is now crucial, especially in view of climate changes and rising world population. Without mitigation actions, modelling indicates that the rise in [$CO_2$] (from current levels of 413 to ~900 μmol $mol^{-1}$) and temperatures predicted by the end of the century are unprecedented and the climate clock may be rewound, thus reversing the evolutionary pressures that have shaped the modern vegetation. In angiosperms, there are evolutionary trends to increased conductance to $CO_2$, water transport and stomatal control, from the basal groups to the most recently derived species. Through the PRIN project EvoPlant ("Evolutionary implications for the development of climate resilient productive plants"), the characterisation of these traits will be carried out and it will allow us to shed light on the selective pressures that have driven plant evolution. In particular major adaptations in leaf gas exchange such as the transition between the two types of stomatal morphology: kidney and dumb-bell. The first ones are found in most plants and are identical to the earliest stomata of 410 Mya; dumb-bell stomata have lower volume to surface area and greater mechanical advantage over surrounding subsidiary cells that allow more responsive adjustment of guard cell turgor with wider stomatal opening, but are found only in Poaceae.
In this work, we examined the leaf anatomy of 13 plant species from lycophytes to dicotyledonous angiosperms, via ferns and gymnosperms. By means of transmitted light microscopy and leaf epidermis impressions we are quantifying stomatal morphology and distribution, to analyse the correlation between leaf anatomy and physiology and stomatal patterning. These measurements will then be correlated with the surface area of the mesophyll cells exposed to the intercellular airspaces, the size of the mesophyll cells, the thickness of the mesophyll, the fraction of the volume occupied by the intercellular airspaces, and the total cross-sectional area of the cells that make up the mesophyll. Data will give us an overview of the most efficient combination of stomata-mesophyll traits for climate change resilience and photosynthetic productivity.

## 6.3 = Contrasting patterns of genetic drift and natural selection affect the evolution of *Phragmites australis* and *Nuphar lutea* populations from northern-central Italy

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Several studies on evolutionary processes summarise the relative role of genetic drift and natural selection for species diversification (1). However, the extent to which both evolutionary forces affect populations depends mainly on the ecological features of the species themselves. Moreover, it is well known how the degree of differentiation varies widely among and within plant species, mirroring many physiological, behavioural, and ecological mechanisms (2). Several studies aimed, therefore, at comparing the phenotypic divergence (Pst) to the differentiation of neutral alleles (Fst) to decipher the relative role of genetic drift and natural selection in species differentiation (Pst-Fst comparison). Following this approach, we assessed and compared for the first time the genetic variation and the leaf trait differentiation of two key macrophytes, *Phragmites australis* and *Nuphar lutea*, across six lake systems in northern-central Italy. We then investigated the relative role of genetic drift and natural selection on leaf trait diversification.

As for genetic structure, *P. australis* results were in line with those observed for other Italian and European populations, while *N. lutea* showed a more complex genetic structure than expected at site level, likely due to its mixed mating system. Both species exhibited wide variability in leaf functional traits within and across sites, highlighting a high phenotypic plasticity. Pst-Fst comparisons showed an overall tendency to directional or divergent selection for *P. australis* and to balanced or stabilizing selection for *N. lutea* populations.

The prevalence of vegetative over generative reproduction leads the populations of *P. australis* to be dominated by a few clones well-adapted to the site condition, and the phenotypes were plastically selected from the environment. Differently, the possible mixed mating system associated with geographical isolation among distant sites could reduce the effect of outbreeding depression and constitutes the genetic basis of adaptiveness in *N. lutea*.

1) K.R. Andrews, J.M. Good, M.R. Miller, G. Luikart, P.A. Hohenlohe (2016) Nat. Rev. Gen., 172, 81-92.
2) D.I. Bolnick, R. Svanbäck, J.A. Fordyce et al. (2003) Am. Nat. 1611, 1-28.

## 6.3 = Functional traits variation in *Crocus etruscus* (Iridaceae)

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Determining how plant functional traits respond to environmental conditions, influencing species distribution and community diversity patterns is a central question in ecology. This topic becomes particularly crucial when managing endangered plants. *Crocus etruscus* Parl., a geophyte endemic to Central Italy, typically grows in various sub-Mediterranean broad-leaved forests. This species is classified as “Near Threatened” (NT) in the National and Global Red List of IUCN and is listed in Annex IVb of the Habitat Directive 92/43/EEC (1). To facilitate conservation and management efforts for *C. etruscus*, we examined its intraspecific variability using a functional trait-based approach associated with the CSR strategy (2). Our aim was to determine whether different forest communities influence its ecological strategy. In this study, we selected 13 populations of *C. etruscus* in different forest communities. Specifically, we analyzed leaf area, specific leaf area, and leaf dry matter content of each population. The CSR strategy of *C. etruscus* was calculated using the spreadsheet provided by (3). Most populations exhibited a "CR/CSR" type, with two populations showing a “SR/CSR” type. These findings underscore the significant influence of both stress and disturbance in these environments, irrespective of the forest community type. Currently, we are conducting vegetation sampling and soil composition analyses to investigate the relationship between plant functional strategies and the environment. Exploring trait variation at the intraspecific level holds promise for developing more targeted management strategies, especially for threatened species.

1) A. Carta, A. Alessandrini, F. Frignani, L. Peruzzi (2010) Informatore Botanico Italiano, 42(1), 47-52.
2) J.P. Grime (2001) Wiley-Blackwell, Chichester, UK.
3) S. Pierce, G. Brusa, I. Vagge, B.E.L. Cerabolini (2013) Functional Ecology, 27, 1002–1010.

## 6.3= Comparative analysis of the structural, compositional, and mechanical properties of the awns of *Pelargonium appendiculatum* and *Erodium gruinum* seeds

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Hygroscopic deformations of plant organs are passive movements which occur along predefined orientations following dehydration of the structure and are actuated in most cases by plants as a dispersal mechanism (1). A source of interest for years for their hygroscopic nature are the appendages (awns) from the mericarp of the beak-like fruit of the Geraniaceae family. These awns are attached to the central column of the fruit and linked at their base to the encapsulated seed. Once detached from the fruit body, they can reversibly and passively coil and uncoil in response to changes in environmental humidity, thus favoring seed dispersal and burial in the soil (2). So far, to explain the observed behavior most studies have focused on investigating the orientation of cellulose microfibrils in the cell wall tissues of the awns from different genera of the Geraniaceae family (3). However, the exact mechanism by which the humidity absorption occurs and how the structural, compositional, and mechanical properties of the tissues in different genera affect the humidity absorption, and thus, how the awn coils, still need investigation. This study proposes an in-depth and comparative characterization of the tissues composing the awns from two genera of the Geraniaceae family, *Pelargonium appendiculatum* (L.f.) Willd and *Erodium gruinum* (L.) L'Hér, for their morphological differences by analyzing the structural changes of the tissues along the length of the awn, and therefore, in the coiling behavior. To reach this goal, we performed an integrative approach by carrying out a morphological and histological characterization with white-light and epifluorescence microscopy, SEM, E-SEM combined with mechanical analysis of the tissues along the awns. Here, we show for the first time that the structural, compositional, and mechanical properties of the awn deeply affect its sensitivity to humidity and, thus, the dispersal strategy actuated by the plants.

1) R. Elbaum, Y. Abraham (2014) Plant Sci., 223, 124-133.
2) Y. Abraham, C. Tamburu, E. Klein, J.W.C. Dunlop, P. Fratzl, U. Raviv, R. Elbaum (2012) J. R. Soc. Interface (2012) 9, 640–647.
3) Y. Abraham, R. Elbaum (2013) New Phytol., 199, 584-594.

## 6.3 = Intraspecific variability of functional traits along an altitude gradient in *Dianthus virgineus*

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Understanding the sources of intraspecific variation in functional traits is crucial for accurately employing these traits in community and ecosystem models. This study aimed to investigate the intraspecific variability of functional traits in *Dianthus virgineus* L. along an altitudinal gradient and in different habitat conditions. Four traits were considered: Plant size, Leaf Area (LA), Specific Leaf Area (SLA), and Leaf Dry Matter Content (LDMC).
LDMC exhibited a linear increase with altitude, while SLA, LA, and Plant Size showed variations not only with altitude but also with local habitat conditions. A progressive increase of LDMC along the altitudinal gradient indicated the capability of coping with the physical and mechanical stresses typical of high altitudes. At lower altitudes, the constant values of LDMC suggest a pre-existing adaptation to these conditions, indicating greater stability and resistance of these plants to the local influences of different habitats.
Moreover, SLA, LA, and Plant size demonstrated distinct patterns along the altitudinal gradient. These traits exhibited a pronounced decrease at higher altitudes, implying potential trade-offs in resource allocation. Notably, at lower altitudes, their trends varied, highlighting complex interactions between environmental factors and trait expression. Populations in partially wooded, shaded, and humid contexts displayed higher SLA, indicating enhanced photosynthetic efficiency but increased vulnerability to mechanical damage. On the contrary, populations in drier habitats had low SLA values without a proportional increment in LDMC, suggesting the presence of localized stress factors that inhibit investment in high-efficiency photosynthetic cellular tissues but are insufficient to promote cellular reinforcement processes.
The considerable variations in trait attributes observed within the same species across contrasting habitats indicate phenotypic adaptation to specific environmental conditions in each location. Among the habitats, light, soil temperature and humidity, and the availability of nitrate emerged as primary drivers influencing the relationships between intraspecific traits and the environment. This study highlights the significance of considering intraspecific variability of functional traits when characterizing a species. LDMC is overall a more robust functional trait than SLA, but using a single trait value to describe a given species can hide large functional variations, especially along an elevation gradient. At the upper end of the altitude gradient, functional traits tend to be more stable among populations. These results collectively suggest that environmental filtering selects locally adapted genotypes within plant species and restricts individuals to specific elevation ranges based on their potential leaf trait values.
Variations in functional traits along the altitudinal gradient provide an understanding of the complex relationships between functional traits, environmental conditions, and plant adaptations. It is crucial to comprehend these dynamics to assess the ecological implications and resilience of species and communities in different habitats.

## 6.5 = The toxic freshwater cyanobacterium *Planktothrix rubescens*: a potential risk for marine coastal environments

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*Plankthothrix rubescens* is a freshwater cyanobacterium able to produce cyanotoxins, mainly microcystins. It can form blooms in water bodies, during which microcystins can reach the sea and potentially can be accumulated in bivalve molluscs posing a risk to consumers. Indeed, in the northern coast of Puglia, high concentrations of microcystins produced by *P. rubescens* were found in mussels, which exceeded safe daily intake levels (1). To investigate the tolerance of *P. rubescens* to increasing salinity and its microcystin production, one strain was isolated from Lake Castreccioni (Cingoli, MC) and exposed to different salinities (0, 10, 20, 30) in batch cultures for a month. Cell abundances and growth rate were obtained by optical density (OD650 e OD750) at the spectrophotometer and by counts through light microscope. Results showed that *P. rubescens* was unable to survive for more than 96 hours at salinity equal to or greater than 10. However, this inhibition was not immediate, and the cells remained viable enough for filter-feeding organisms to accumulate cells and toxins in their tissues. This implies that once the cells have reached the coasts, they remain vital enough to allow filter-feeding organisms to accumulate cells and toxins in their tissues. Toxins were quantified in both the intracellular compartment and the culture medium using LC-MS/MS, with the most common microcystin being MC-RR desmethylated. Higher intracellular concentrations of microcystins were observed at lower salinities (0 and 10), while at higher salinities (20 and 30) the amount of dissolved toxins increased and remained stable until the end of the experiment. These findings suggest that salinity stress could cause plasmolysis, as already found by Rosen et al. (2), and the release of toxins. Moreover, it seems that high salinity could keep microcystins' levels stable. Therefore, it is crucial to monitor cyanotoxins in seawater and bivalve molluscs for public health purposes.

1) R. De Pace, V. Vita, M. Bucci, P. Gallo, M. Bruno (2014) Journal of Ecosystems, 2014, Article ID 374027.
2) B. H. Rosen, K. A. Loftin, J. L. Graham, K. N. Stahlhut, J. M. Riley, B. D. Johnston, S. Senegal (2018) 2018–5092. US Geological Survey.

## 6.6 = Monitoring of two forests damaged by the Vaia storm reveals a gradual restoration of vegetation greenness and possible changes in mature forest communities

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Windstorms are rare in the Southern Alps, but their frequency is increasing due to climate change. Vaia storm hit the Italian Alps from 28 to 30 October 2018 (with winds exceeding 200 km $h^{-1}$ and strong rainfall) and caused extensive forest damage in 494 Italian municipalities. Camonica Valley (Northern Italy, Lombardy region) was one of the most affected areas, with about 800 ha of forests (mostly spruce forests) devastated, and 300,000 $m^3$ of downed trees.
This research analyzed the vegetation of two spruce forests located in Camonica Valley destroyed by the Vaia storm (Fig. 1) to evaluate the vegetation responses to blowdown damage (1). In each study area, the normalized difference vegetation index (NDVI) was used to evaluate the change in plant cover and greenness from 2018 (before the Vaia storm) to 2021. Furthermore, floristic-vegetational data were collected (according to the phytosociological method of Braun-Blanquet) and analyzed to identify current plant communities and develop models of plant succession.
The results showed that the two areas, although located in different altitudinal vegetation belts, are undergoing the same ecological processes. NDVI is increasing in both areas and pre-disturbance values (~0.8) will be reached in less than ten years. Nevertheless, the spontaneous restoration of pre-disturbance forest communities (*Calamagrostio arundinaceae-Piceetum*) does not appear to occur in both study areas. In fact, the two plant succession models are characterized by pioneer stages (mainly *Rubetum idaei*) and intermediate stages (shrubs of *Astrantio-Corylion avellanae* and *Piceo abietis-Sorbetum aucupariae* alliances) with young trees (*Quercus petraea* and *Abies alba*) typical of more thermophilic mature forest communities than pre-disturbance ones. These results indicate that also in Camonica Valley there is an upward shift in forest plant species caused both by global warming and by the different intensity of forest use and management compared to the past.

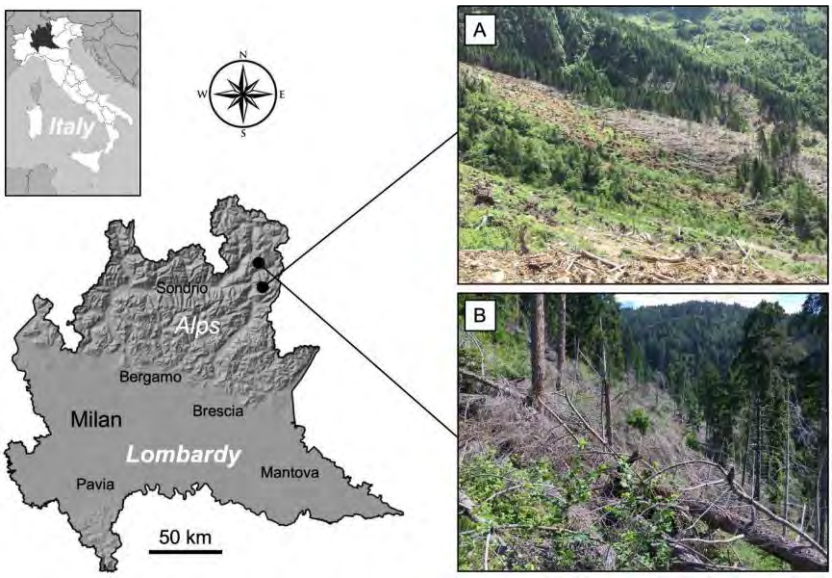


Fig. 1. Location of the two study areas: Val Malga (A) (high mountain belt) and Vione (B) (mountain belt). The two study areas are part of the acidophilic alpine series of silver fir and spruce (*Calamagrostio arundinaceae-Piceo excelsae sigmetum*). The photographs show the areas in July 2021, when phytosociological relevés were carried out.

1) L. Giupponi, V. Leoni, D. Pedrali, A. Giorgi (2023) Plants, 12, 1369.

**Elenco completo degli abstract in ordine alfabetico**