UNIVERSITÀ DEGLI STUDI FIRENZE

PhD IN
AGRICULTURAL AND ENVIRONMENTAL SCIENCES

CYCLE XXXVIII

# Circular agroecology practices for soil health and valorisation of urban and rural wastes

Academic Discipline (SSD) AGRI-02/A

**Doctoral Candidate**
Dr. Francesco Serafini

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# Contents

# 1 Abstract

Climate change, lack of water, higher temperatures, and rising energy costs represent major and interconnected threats facing Mediterranean agriculture. Increasing climatic variability, more erratic rainfall, and frequent extreme events are reducing the reliability of rain-fed cropping systems, while the recent energy crisis is making input-intensive practices increasingly difficult to sustain. In this context, the integration of agroecological management with circular economy strategies has been proposed as a means to restore soil ecosystem functions, support crops through the recycling of locally available organic wastes, and improve energy efficiency.

This thesis evaluates the potential of this integrated approach through a compendium of complementary studies. First, a 30-year analysis of the Montepaldi Long-Term Experiment[1] reveals that both organic and conventional farming systems experienced a significant yield decline consistent with a worsening water balance; however, organic management maintained substantially higher energy use efficiency despite lower winter cereal yields. Second, a systematic review of Municipal Solid Waste Compost (MSWC) confirms that its application can increase crop yields and soil organic matter content, with the most consistent benefits observed at high application rates, while highlighting strong variability in waste stream composition and composting procedures, as well as the potential introduction of contaminants such as heavy metals and microplastics. Third, a two-year field trial on winter cereals at MoLTE tested a realistic repeated application rate (2 t $ha^{-1}$ $yr^{-1}$, corresponding to the most common regional availability) of composted vine prunings and vermicompost derived from spent coffee grounds, resulting in improved soil aggregate stability and a yield increase in the second year, while emphasizing the need for explicit phosphorus management. Fourth, a mesocosm study assessed behavioral and functional responses of the earthworm *Hormogaster samnitica*, the most abundant species at MoLTE, demonstrating size-dependent functional roles: larger individuals created wider and deeper macropores with potential implications for water infiltration; composted vine prunings were associated with increased earthworm activity and macroporosity in amended soil layers compared to vermicompost; a two-choice preference test revealed weak and poorly differentiated substrate selection by *Hormogaster samnitica*, whereas the more generalist species *Aporrectodea caliginosa* showed a clear preference for compost-based amendments over vermicompost and the control. Finally, a pot experiment investigated dose-dependent responses of substrates and plants to vermicompost and pruning-based composts, showing that vermicompost enhanced both substrate water retention and lettuce growth even at modest application rates, whereas lignin-rich pruning composts produced weaker short-term agronomic responses.

[1]MoLTE, https://www.molte.unifi.it

Overall, these findings indicate that organic farming, when complemented with context-appropriate amendments derived from the circular economy, can improve energy use efficiency and selected soil functions under Mediterranean constraints. Maximizing these benefits will require aligning amendment type and application rate with local biomass availability, explicitly managing phosphorus inputs, standardizing compost quality, and integrating soil fauna metrics, alongside the development of crop germplasm adapted to water-limited, low-input systems.

# 2 General Introduction

## 2.1 Background

The Mediterranean region stands at the forefront of environmental challenges, exacerbated by the intensifying impacts of climate change. Rising temperatures, irregular rainfall patterns, and an increasing frequency of extreme events are already reshaping ecosystems and agricultural practices across the area (Intergovernmental Panel On Climate Change (Ipcc) 2023; Lionello and Scarascia 2018). These climatic pressures are projected to worsen in the coming decades, with droughts becoming longer and more severe, putting rain-fed cropping systems at particular risk. Projections indicate yield reductions by up to 64% in some locations, primarily due to water shortages during critical phenological stages of crop growth (Intergovernmental Panel On Climate Change (Ipcc) 2023).

The term “Perfect Storm” has been used to describe the looming coincidence of food, water, and energy insecurity (Godfray et al. 2010). In the Mediterranean, this concept is particularly fitting: climate change interacts with structural weaknesses in soil and water resources, creating a hotspot for highly interconnected risks. Attempts to compensate for declining yields by intensifying inputs — through chemical fertilizers, xenobiotics, or irrigation — are increasingly untenable. The ongoing global energy crisis, defined as a “shock of unprecedented breadth and complexity” (IEA 2022), no longer allows for a massive reliance on high-energy inputs. Thus, solutions based on input-intensive agriculture appear unsustainable in this region.

Thus, agroecology has gained recognition as a scientific and practical framework capable of addressing those converging crises. Rooted in ecological principles, agroecology promotes the recycling of nutrients, diversification of cropping systems, and the valorization of local resources while reducing dependence on external inputs (Altieri et al. 2018; FAO 2018; Gliessman 2014). By reintroducing organic matter into soils, fostering functional biodiversity, and strengthening ecosystem services, agroecological systems are better than conventional ones at mitigating climate variability and sustaining productivity in resource-constrained environments. Organic agriculture — one of the most consolidated expressions of agroecology, particularly with respect to the application of agroecological principles such as nutrient recycling, soil organic matter management, and reduced reliance on external synthetic inputs — has been repeatedly proposed as a viable alternative under adverse future scenarios (Altieri et al. 2018; FAO 2018; Gliessman 2014). Both modeling studies and long-term comparisons suggest that organic systems can achieve acceptable levels of food production while consuming fewer resources and enabling greater energy efficiency (Maeder et al. 2002; Muller et al. 2017; Poux and Aubert

2018).

However, we must not forget that Mediterranean agroecosystems face structural barriers that complicate the transition to agroecological/organic management. A central issue here is the persistent scarcity of soil organic matter (SOM), which in many arable soils remains around or below 1–1.5% (Lal 2020). Its depletion reduces soil fertility, weakens particle aggregation, lowers water retention capacity, and compromises the biological activity that underpins nutrient cycling (Lal 2004). The integration of crops and livestock ensured a regular return of organic inputs to soil in the past (Manlay et al. 2007), but decades of agricultural intensification and specialization have disrupted this cycle. Livestock are currently concentrated in limited areas, while stockless arable systems dominate elsewhere (Canali et al. 2005; Feller et al. 2012). As a result, farmers are often forced to rely on external organic inputs, a process sometimes described as the "conventionalization" of organic farming (Darnhofer et al. 2010). To address the effect of low SOM, due the separation between crop and livestock within Mediterranean agroecosystems, circular economy proposes some possible remedies such as the amendment of soils with composted and vermicomposted locally-available materials, such as pruning residues, agro-industrial by-products and urban organic waste. This waste management can recycle carbon and nutrients back into soils, can close cycles locally, and can reduce the environmental impact of unmanaged residues (Bernal et al. 2009; FAO 2018). Beyond their role in nutrient supply, such amendments influence soil physical and biological properties: compost improves aggregation and cation exchange capacity, while vermicompost, often finer and microbially enriched, can stimulate plant growth and soil biota at relatively modest doses (Dominguez and Edwards 2010; Lazcano and Dominguez 2011).

Yet, agroecosystems are highly complex. Their functioning depends on multiple interacting drivers — such as climatic conditions, soil type, crop species, management practices, biological communities — resulting in responses that are both site-specific and time-dependent (Conway 1987; Marten 1988). Evaluating with experiments the impact of farming practices within this complexity is not a trivial task, thus robust and stable experimental settings such as those intrinsically present in Long-Term Experiments (LTEs) are necessary. They provide an invaluable framework, generating a continuous data flow over decades, capturing both slow soil processes and cumulative management effects that shorter trials cannot reveal (Potschin and Haines-Young 2011).

At the other end of the spectrum of possible types of experiments, we find methods such as pot experiments, mesocosms, and short-term field trials, which remain nevertheless essential to disentangle mechanistic pathways, such as structural changes in the soil, the role of soil fauna, or plant physiological responses.

## 2.2 Problem Statement

Despite some promising evidences on the greater energy use efficiency of organic farming compared to conventional one, Mediterranean agriculture remains constrained by water scarcity and declining soil fertility (Intergovernmental Panel On Climate Change (Ipcc) 2023; Montanarella et al. 2016).

The productivity of organic systems is often penalized by lower yields, particularly in winter cereals, while their resilience to climatic extremes is not yet fully understood (Ponisio et al. 2015; Seufert et al. 2012). Furthermore, the distribution of organic amendments — a cornerstone of organic farming — is hampered by several factors. First, the composition of organic amendments is inconsistent, depending on feedstock origin and processing methods. This lack of homogeneity is particularly evident in Municipal Solid Waste Compost (MSWC), where differences in waste stream composition and composting procedures generate inconsistent agronomic performances. Second, many studies indicate the need of high application rates to achieve significant improvements either in yield or soil fertility (Diacono and Montemurro 2010), yet such rates are often neither logistically feasible nor economically viable in stockless organic farms, where the availability of residues is limited. Third, the potential presence of contaminants such as heavy metals, microplastics, or Persistent Organic Pollutants (POPs) in either MSWC or agro-industrial residues, raises concerns about long-term soil health and crop safety, issues currently lacking from literature (Belén Almendro-Candel et al. 2019).

Moreover, the functional role of soil biota in mediating the effects of amendments remains underexplored in Mediterranean contexts. Earthworms, for instance, are considered "ecosystem engineers" that strongly influence soil structure, porosity, and infiltration through bioturbation (Bertrand et al. 2015). Their behavioral and functional responses are not only indicators of amendment's quality but also determinants of amendment's efficacy. As a case in point, the endemic Mediterranean earthworm *Hormogaster samnitica* remains scarcely investigated, despite its potential relevance for region-specific responses.

Therefore, there is a need for an integrated evaluation that connects: i) system-level energy-efficiency versus yield trade-offs under organic vs conventional management, ii) soil responses to locally sourced amendments at both high and realistic or economically feasible low rates, iii) the role of soil fauna as mediators and indicators of soil functional recovery.

## 2.3 Objective of the Research

This work is an attempt to combine the complementary roles of LTEs and targeted studies within an agroecological framework, here intended as the integration of (i) reduced dependence on external and energy-intensive inputs, (ii) recycling of locally available biomass through circular waste management to restore soil organic matter and soil functions, and (iii) explicit consideration of functional biodiversity, including soil fauna, as both an indicator and a driver of ecosystem services. It investigates the effects on soil fertility and crop performance following repeated applications of organic amendments derived from circular waste management in Mediterranean organic farming systems. Special attention is given to amendments obtained from locally available resources, such as: *i) composted vine prunings and ii) vermicompost from spent coffee grounds*. This work contributes to understanding how agroecological principles can be put into practice to address the intertwined issues of climate, energy, and soil degradation in Mediterranean agriculture.

More specifically, it aims to:

- quantify long-term trends in yield and energy use efficiency in organic and conventional arable systems under Mediterranean conditions, using data from the Montepaldi Long-Term Experiment (MoLTE, Italy);

- summarize scientific evidence on the agronomic impacts of MSWC on crop productivity and soil fertility, identifying consolidated findings, uncertainties, and research gaps through a systematic review;

- evaluate the agronomic value of small, yet realistic and economically feasible, repeated field applications of the composts listed above in organic farming systems (winter cereals), with a focus on selected soil chemical, physical, and biological properties, as well as crop yield;

- investigate, in a mesocosm experiment, the behavioral and functional responses of the endemic earthworm *Hormogaster samnitica* present at MoLTE to the amendments listed above, clarifying how amendment type and earthworm traits influence soil porosity and water infiltration;

- assess short-term, dose-dependent effects of the same amendments under controlled pot conditions, with particular attention to soil water retention, salinity, and plant growth responses and characteristics at harvest.

These objectives are complementary and collectively address the urgent need to reconcile productivity, soil fertility, and circularity in Mediterranean arable agriculture.

## 2.4 Outline of the Thesis

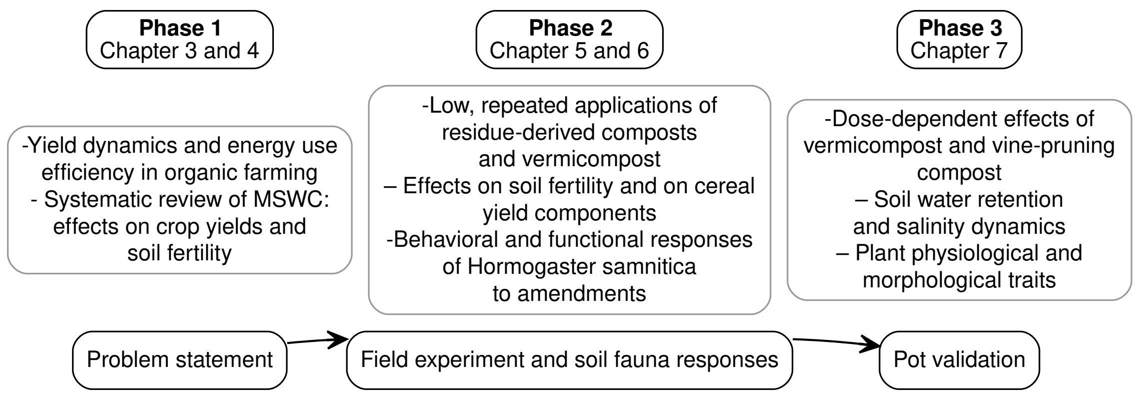


Figure 1: Research outline.

Chapter 3 presents the paper *Mediterranean Climate Change: Is Organic Agriculture an Option to Face a Perfect Storm?*, which analyses yield dynamics and energy use efficiency in organic and conventional farming systems by data collected since 1992 from the Montepaldi Long-Term Experiment (MoLTE). The analysis considers major staple crops cultivated under rainfed conditions, linking yield trends with climatic variables and energy balances.

Chapter 4 focuses on the paper *A Systematic Review of Scientific Research on Municipal Solid Waste Compost: Impacts on Crop Productivity and Soil Fertility*. This review summarizes global scientific evidences on the effects of MSWC application on crop yields and soil fertility. It highlights consolidated findings, uncertainties, and research gaps, and discusses the implications of these results for Mediterranean contexts.

Chapter 5 reports the paper *Circular Soil Amendment Strategies in Mediterranean Organic Systems: A Two-Year Field Study in the Montepaldi Long-Term Experiment, Tuscany*, where are presented the results of a field trial on repeated applications of realistic amounts of composted vine prunings and vermicompost from spent coffee grounds, assessing their effects on soil chemical, physical, and biological indicators as well as on winter cereals yields.

Chapter 6 contains *Behavioral and Functional Responses of Hormogaster samnitica to Different Organic Amendments*, a paper describing the impact on soil porosity and water infiltration from a mesocosm study on the burrowing behavior and functional role of the endemic Mediterranean earthworm *Hormogaster samnitica*, the most abundant species at the MoLTE, under amendments of composted vine prunings and vermicompost from spent coffee grounds.

Chapter 7 presents the paper *Plant Response in Soil Amended With Vermicompost and Composted Vine Pruning: A Pot Experiment*. This study evaluates short-term responses of soil and plants under monitored pot conditions, where the realistic doses of the very same composts were compared against high doses as found in Chapter 4. Soil water retention and salinity, as well as physiological and morphological traits of *Lactuca sativa* were measured and compared.

Chapter 8 synthesizes the main findings of the thesis, integrating results across long-term experiments, field trials, mesocosm studies, and pot experiments. This chapter discusses the broader implications of the research for agroecological management, circular economy strategies, and climate resilience in Mediterranean agriculture, and outlines key perspectives for future research.

Supplementary Materials, Chapter 9, illustrates the *FunBies* model (*Fun*ctional *Bi*odiversity of Agro-*E*cosystem*s*), an approach for the integrated assessment of the functional biodiversity of weed communities. The application of this model to the MoLTE dataset is described, with results quantifying the ecosystem services provided by weed communities in organic and conventional farming systems.

## 2.5 References

Altieri MA, Farrell JG, Hecht SB, Liebman M, Magdoff F, Murphy B, Norgaard RB, Sikor TO (2018) Agroecology: The Science of Sustainable Agriculture, 2nd edn. CRC Press

Belén Almendro-Candel M, Navarro-Pedreño J, Gómez Lucas I, A. Zorpas A, Voukkali I, Loizia P (2019) The Use of Composted Municipal Solid Waste under the Concept of Circular Economy and as a Source of Plant Nutrients and Pollutants. In: El-Din Mostafa Saleh H (ed) Municipal Solid Waste Management. IntechOpen

Bernal MP, Alburquerque JA, Moral R (2009) Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresource Technology 100:5444–5453. https://doi.org/10.1016/j.biortech.2008.11.027

Bertrand M, Barot S, Blouin M, Whalen J, de Oliveira T, Roger-Estrade J (2015) Earthworm services for cropping systems. A review. Agronomy for Sustainable Development 35:553–567. https://doi.org/10.1007/s13593-014-0269-7

Canali S, Stopes C, Schmid O, Speiser B (2005) Current evaluation procedures for fertilizers and soil conditioners used in organic agriculture. Proceedings of a workshop held april 29–30, 2004 at emerson college, great britain. 100

Conway GR (1987) The properties of agroecosystems. Agricultural Systems 24:95–117. https://doi.org/10.1016/0308-521X(87)90056-4

Darnhofer I, Lindenthal T, Bartel-Kratochvil R, Zollitsch W (2010) Conventionalisation of organic farming practices: From structural criteria towards an assessment based on organic principles. A review. Agronomy for Sustainable Development 30:67–81. https://doi.org/10.1051/agro/2009011

Diacono M, Montemurro F (2010) Long-term effects of organic amendments on soil fertility. A review. Agronomy for Sustainable Development 30:401–422. https://doi.org/10.1051/agro/2009040

Dominguez J, Edwards C (2010) Relationships between Composting and Vermicomposting. In: Edwards C, Arancon N, Sherman R (eds) Vermiculture Technology. CRC Press, pp 11–25

FAO (2018) The 10 Elements of Agroecology. Guiding the Transition to Sustainable Food and Agricultural Systems.

Feller C, Blanchart E, Bernoux M, Lal R, Manlay R (2012) Soil fertility concepts over the past two centuries: The importance attributed to soil organic matter in developed and developing countries. Archives of Agronomy and Soil Science 58:S3–S21. https://doi.org/10.1080/03650340.2012.693598

Gliessman SR (2014) Agroecology: The Ecology of Sustainable Food Systems, Third Edition, 0th edn. CRC Press

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food Security: The Challenge of Feeding 9 Billion People. Science 327:812–818. https://doi.org/10.1126/science.1185383

IEA (2022) International energy agency (IEA), 2022. World energy outlook 2022. paris. License: CC BY 4.0 (report); CC BY NC SA 4.0 (Annex A):

Intergovernmental Panel On Climate Change (Ipcc) (2023) Climate Change 2022 – Impacts, Adaptation and Vulnerability: Working Group II Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change, 1st edn. Cambridge University Press

Lal R (2020) Soil organic matter content and crop yield. Journal of Soil and Water Conservation 75:1. https://doi.org/10.2489/jswc.75.2.27A

Lal R (2004) Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. Science 304:1623–1627. https://doi.org/10.1126/science.1097396

Lazcano C, Dominguez J (2011) The use of vermicompost in sustainable agriculture: Impact on plant growth and soil fertility. In: Soil nutrients

Lionello P, Scarascia L (2018) The relation between climate change in the Mediterranean region and global warming. Regional Environmental Change 18:1481–1493. https://doi.org/10.1007/s10113-018-1290-1

Maeder P, Fliessbach A, Dubois D, Gunst L, Fried P, Niggli U (2002) Soil Fertility and Biodiversity in Organic Farming. Science 296:1694–1697. https://doi.org/10.1126/science.1071148

Manlay RJ, Feller C, Swift MJ (2007) Historical evolution of soil organic matter concepts and their relationships with the fertility and sustainability of cropping systems. Agriculture, Ecosystems & Environment 119:217–233. https://doi.org/10.1016/j.agee.2006.07.011

Marten GG (1988) Productivity, stability, sustainability, equitability and autonomy as properties for agroecosystem assessment. Agricultural Systems 26:291–316. https://doi.org/10.1016/0308-521X(88)90046-7

Montanarella L, Pennock DJ, McKenzie N, et al (2016) World's soils are under threat. SOIL 2:79–82. https://doi.org/10.5194/soil-2-79-2016

Muller A, Schader C, Scialabba NE-H, Bruggemann J, Isensee A, Erb K-H, Smith P, Klocke P, Leiber F, Stolze M, Niggli U (2017) Strategies for feeding the world more sustainably with organic agriculture. Nature Communications 8:1290. https://doi.org/10.1038/s41467-017-01410-w

Ponisio LC, M'Gonigle LK, Mace KC, Palomino J, De Valpine P, Kremen C (2015) Diversification practices reduce organic to conventional yield gap. Proceedings of the Royal Society

B: Biological Sciences 282:20141396. https://doi.org/10.1098/rspb.2014.1396

Potschin MB, Haines-Young RH (2011) Ecosystem services: Exploring a geographical perspective. Progress in Physical Geography: Earth and Environment 35:575–594. https://doi.org/10.1177/0309133311423172

Poux X, Aubert P-M (2018) An Agroecological Europe in 2050: Multifunctional Agriculture for Healthy Eating: Findings from the Ten Years For Agroecology (TYFA) Modelling Exercise. Institut du Développement Durableet des Relations Internationales (IDDRI)

Seufert V, Ramankutty N, Foley JA (2012) Comparing the yields of organic and conventional agriculture. Nature 485:229–232. https://doi.org/10.1038/nature11069

# 3 Mediterranean Climate Change: Is Organic Agriculture an Option to Face a Perfect Storm?

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## 3.1 Abstract

The current climate, energy and food crises require a reflection on which agricultural production systems can maintain productivity while reducing energy demand. This is particularly relevant in the Mediterranean region, a hotspot for highly interconnected environmental risks. Against this background, assessing to what extent organic agriculture can cope with adverse climatic and energetic scenarios under Mediterranean conditions becomes a key research question. We analysed data from the Montepaldi Long-Term Experiment (MoLTE, Tuscany, Italy), where stockless organic and conventional arable farming systems have been running since 1992 under rainfed conditions. Using a 30-year time series (1993–2022), we quantified yield dynamics and investigated their relationships with climatic variables and soil parameters. In addition, we performed an energy analysis to estimate energy inputs and outputs and calculate energy use efficiency (EUE) for the main crops cultivated simultaneously in both systems.

Yields significantly decreased with time in both systems (about -79% and -37% since the beginning of the experiment for spring and winter crops, respectively), most likely due to reduced cumulative rainfall from seeding to harvesting. Organic winter crops yielded approximately 21% less than conventional crops, while spring crops did not show significant differences between systems. Despite lower winter crop yields, organic management showed higher EUE than conventional management: +33% for winter crops and +44% for spring crops. Overall, under increasing climate change impacts and rising energy constraints, the organic system maintained higher energy performance, suggesting that organic management can represent a viable option to improve the energy-efficiency versus yield trade-off in Mediterranean rainfed agroecosystems.

## 3.2 Keywords

Organic agriculture; Conventional agriculture; Long Term Experiment; Mediterranean area; Climate change; Energy balance

## 3.3 Introduction

The term "perfect storm" has been used to describe the future coincidence of food, water, and energy insecurity (Godfray et al. 2010). Due to the combination of its peculiar climate hazards and high soil vulnerability, the Mediterranean area stands out as a hotspot for highly interconnected environmental risks. Climate change poses a threat to water availability, potentially leading to a 64% decrease in yields of rain-fed crops in certain locations, primarily due to more frequent droughts(Ali et al. 2022), with extreme events also in the current agricultural campaign leading to, e.g. in Southern Italy, an estimated 70-90% loss in wheat yields. Therefore, compensating for the lack of water through irrigation appears to be the sole adaptation strategy to climate change in the Mediterranean area. However, from a sustainability perspective, the current global energy crisis, recently defined as a shock of unprecedented breadth and complexity(IEA 2022), no longer allows for the massive use of high-energy inputs such as chemical fertilizers, pesticides, and irrigation. Amidst growing energy and climate emergencies, understanding which agricultural production system performs better in terms of energy consumption becomes crucial. Several modelling studies have promoted the idea of organic farming as a viable option to face future adverse scenarios, mostly because of its capacity to achieve satisfying levels of food production while consuming fewer resources (Mäder et al. 2002; Muller et al. 2017; Poux and Aubert 2018). However, further efforts are needed to understand to what extent organic agriculture can cope with adverse scenarios, given different pedologic, climatic and agronomic conditions. The inevitable multiple interactions among these factors, over medium and long-term spans and spatial scales, perfectly summarize the complexity in the analysis of agroecosystems (Altieri 1996; Conway 1987; Gliessman 2006; Marten 1988). Agroecosystems are characterized by a broad spectrum of interacting drivers that impact a large number of components and processes including functional biodiversity, energy flows, biogeochemical cycles, and interactions between organisms and biotopes. Considering these aspects, the evaluation of the impacts of farming practices becomes highly complex. To elucidate these intricate interactions, it is necessary to consider the results from specifically designed long-term experiments (LTE), where continuous recording of data ensures a more comprehensive explanation of the long-term effects of agricultural practices. The presence of LTE is particularly necessary when solutions are sought within a sustainability choice space (Potschin-Young and Haines-Young 2011) restrained by severe environmental and productive conditions, as is currently happening in the Mediterranean region. Here, farmers have limited technical and agronomic options due to arid conditions, prolonged droughts, and scarce levels of water retention, most probably due to low levels of organic matter in soils which often hover around 1.5%(Altobelli and Piazza

2022).

Backed by all these considerations, we have analysed the data recorded in the Montepaldi Long Term Experiment, MoLTE (Tuscany, Italy), the most enduring LTE of the Mediterranean area[2]. Organic and conventional rain-fed systems were established in 1992 and they have continued to run since then. The present study, covering the period from 1993 to 2022, considers the relationships between crop yields and other variables such as climatic data, soil parameters, and energy use. However, to conduct a comparative analysis between the two systems, the focus was restricted to the data from 1994 to 2017 as during this period a subset of crops was simultaneously sown in both systems. Therefore, we present here the results regarding the main staple non-irrigated crops such as common and durum wheat, barley, maize, and sunflower. This is the first time that agronomic, climatic and energy data have been shown considering a 30-year dataset in a long-term experiment located in the Mediterranean area.

[2]https://www.dagri.unifi.it/vp-475-molte.html?newlang=eng

## 3.4 Material and Methods

### 3.4.1 Description of the Montepaldi Long Term Experiment (MoLTE)

The Montepaldi Long Term Experiment (MoLTE) has been active since 1992 at the experimental farm of the University of Florence (location Montepaldi, San Casciano Val di Pesa, Italy, Long. 11°09'08' ' E, Lat. 43°40'16' ' N, 90 m a.s.l). This experiment is unique in Italy and throughout the Mediterranean area for its duration and amount of data collected. The field experiment encompasses a slightly sloping surface of about 15 ha. Each individual plot measures 1.3 ha, with a total of 10 plots. The main soil physico-chemical characteristics at MoLTE are shown in Table S1. Two stockless arable systems have been established since 1992, primarily differing in fertilization strategy and herbicide usage:

1. an organic system, certified as organic agriculture since 1992 (EC reg. 2092/91 and following regulations), where organic fertilizers, amendments and green manure have been used;
2. a conventional/high-input system, where xenobiotics and synthetic fertilizers have been routinely applied since 1992.

The typical fertilization intensity found on ordinary organic and conventional farms in the region has been applied at MoLTE. Both organic and conventional systems abstain from disease and pest control measures, in line with ordinary farms in the region. The sole method of protection carried out was seed treatment, using copper salts in the organic system and fungicides in the conventional one. Additional agronomic details can be found in Table S2. To minimize the risk of interactions and cross-contamination, natural and artificial hedges have been interposed between the two systems since 1992. Crop rotations, outlined in Table S3, differ between the organic and conventional systems. Since 1992, the organic system has adhered to a four-year rotation, while the conventional system has employed a two-year rotation. Rotations changed six times from 1992 to 2022, depending on the research focus in each period. For the purposes of the present study, we specifically compared the performances of winter and spring crops simultaneously cultivated in both systems. Thus, the crops under analysis cover the period from 1994 to 2017 and include *Hordeum vulgare* L. (BA), *Triticum aestivum* L. (WC), *Triticum durum* L. (WD), *Zea mays* L. (MA) and *Helianthus annuus* L. (SU), as detailed in Table S4, which also indicates the number of yield observations for each crop over the years. Furthermore, average yields for each crop estimated by a linear model are presented in Table S5.

### 3.4.2 Climate

The experimental site is characterized by a typical Mediterranean and Sub-Apennine climate with an average annual rainfall of 886 mm and a mean annual temperature of 15 °C. Daily recordings of maximum and minimum temperatures, as well as rainfall, were obtained from two weather stations during two distinct periods:

1. station "San Casciano Val di Pesa" - Lat. 43°40'11.0"N, Long. 11°09'05.0"E, 230 m a.s.l - from 1993/07/01 to 2015/05/20;

2. station "Sambuca" - Lat. 43°35'41.9"N, Long.11°14'03.3"E, 325 m a.s.l - from 2001/01/23 to 2022/11/20.

Common dates to both weather stations showed no significant differences in the described climate variables. Consequently, data from 1993/07/01 to 2015/05/20 were selected from station 1), while those from 2015/05/21 to 2022/11/20 were selected from station 2).

Figure S1 displays trends over the years for maximum temperature and rainfall, processed using 'stl' function (Cleveland et al. 1990). An increase in maximum temperatures by 1 °C and a decrease in rainfall by 167 mm from 1993 to 2022 were recorded.

### 3.4.3 Soil parameters

From 1994 to 2017, the following chemical indicators were measured at MoLTE: soil organic matter(Walkley and Black 1934), total N content(Kjeldahl 1883), available $P_2O_5$(Olsen et al. 1954), and exchangeable $K_2O$. In Table S6, the number of observations for the main soil chemical analysis in organic and conventional systems is reported.

### 3.4.4 Energy balance

The energy aspects of organic and conventional systems were evaluated by an energy analysis, providing a means to compare farming systems and compute their energy balance (Hülsbergen et al. 2001; Lin et al. 2016). The energy parameters were calculated according to Table S7. In particular, the systems were assessed in terms of Energy inputs ($E$), Energy outputs ($Eo$), and Energy Use Efficiency ($EUE$). Widely-used conversion coefficients, known as energy equivalents, were used to calculate each energetic parameter (Table S8).

### 3.4.5 Statistical analyses

Statistical analyses were performed using R statistical software (version 4.3.2; R Core Team (2023)) and several of its libraries (Baker and Mortlock 2023; Dowle and Srinivasan 2023; Fox et al. 2023; Mayer 2023; Ryan and Ulrich 2023; Sarkar 2008, 2023; Sax 2023; Spinu et al. 2023; Wickham 2023). Crop yield, soil, climatic, and energy variables were analysed using linear regression models, including multiple linear regression where appropriate, to quantify the effects of management system (organic vs conventional), time (year), and selected climatic covariates, as well as their interactions. Models were fitted using the `lm()` function.

For each response variable, an initial saturated model was specified, including all relevant predictors. Model selection was performed by sequentially removing non-significant terms and comparing nested models, retaining the most parsimonious model that adequately explained the data. The significance of model terms was assessed using t-tests for regression coefficients and F-tests for overall model effects, with a significance threshold of $p < 0.05$.

Model assumptions were evaluated through residual diagnostics, including normality and homoscedasticity. The residuals of the final models did not show substantial deviations from these assumptions. Statistical significance reported in figures and tables is based on the results of the final selected linear models.

## 3.5 Results and discussion

### 3.5.1 Relationships between agronomic aspects and climate

The relationships between yields and time are presented in Figure 2. Yields significantly decreased over the years in both systems. For winter crops, the decrease fluctuation was -42% (from 3.8 to 2.2 ton ha$^{-1}$) in the organic system and -33% (from 4.8 to 3.2 ton ha$^{-1}$) in the conventional one. In contrast, for spring crops, there was a substantial reduction (-79%, from 2.4 to 0.5 ton ha$^{-1}$) in both systems.

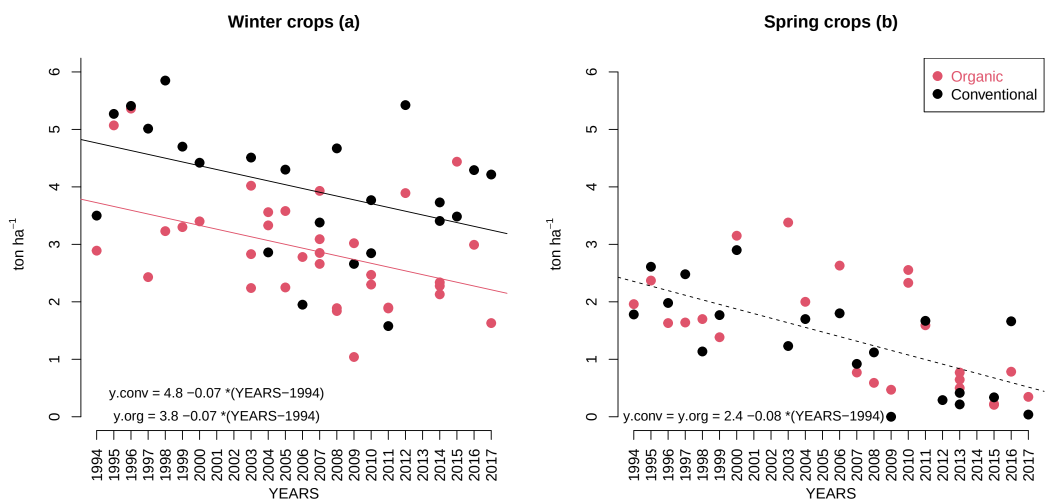


Figure 2: Yields in the organic and conventional systems for winter (a) and spring (b) crops at MoLTE. Only the crops cultivated simultaneously in both systems were considered. Dotted line represents no significant difference between systems (p 0.05).

Regarding climatic variables, a significant decrease in cumulative rainfall during the winter and spring crops' vegetative cycle was observed (Figure 3), associated with an increase of about 1°C in daily maximum temperatures (Figure S1). Therefore, the reduction in yields recorded at MoLTE may be attributed to a significant decrease in cumulative rainfall during the winter and spring crops' vegetative cycle (Figure 3).

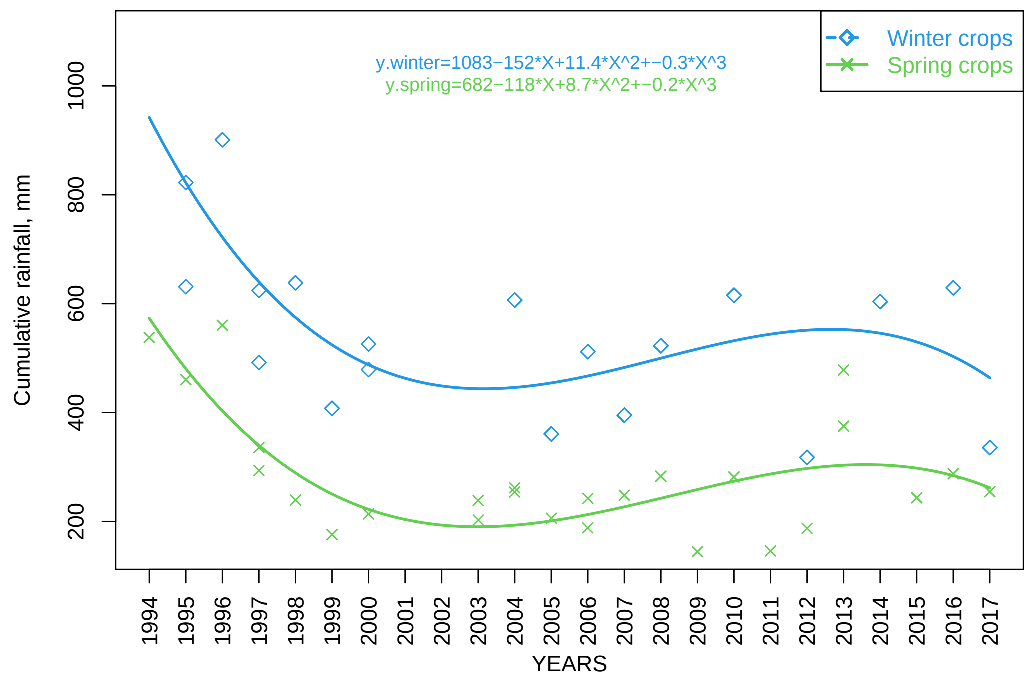


Figure 3: Cumulative rainfall (mm) during the vegetative cycle of winter and spring crops at MoLTE. In the equation, X stands for (YEARS − 1994). Only the crops cultivated simultaneously in organic and conventional systems were considered.

Furthermore, the sowing date for winter crops was found to advance by approximately 32 days, from around November $3^{th}$ in 1994 (DOY 307) to December $5^{th}$ in 2017 (DOY 339), while no difference was observed for spring crops (S2). The delay in the winter crop sowing date probably reflects a decision by agronomists who deemed the climatic and soil conditions unsuitable for sowing in the customary period.

In order to exclude the potential impact of the delayed sowing date on the cumulative rainfall, the vegetative cycle was simulated with the winter crop sowing date held constant at October $25^{th}$, i.e. the earliest date recorded at MoLTE. Nevertheless, a decrease in cumulative rainfall was observed (S3). As predicted by several authors (Bird et al. 2016; Bouregaa 2019; Saadi et al. 2015; Waha et al. 2017), under a warming of 1.5-3°C and a reduced rainfall, the shortening of the crop growing season by up to 30 days could result in a yield decrease in maize

(Georgopoulou et al. 2017; Iocola et al. 2017) and barley (Bouregaa 2019; Cammarano et al. 2019). Hence, the observed delay in sowing, coupled with both the increase in temperature and the decrease in rainfall, might have contributed to the crop yield drop.

The difference in crop yield between the two systems are presented in Figure 2. Since the two systems share a common slope for winter crop yields (-0.07 ton $ha^{-1}$ per year), it is convenient to consider the mean values in 1994, which show that organic winter crops yielded 3.8 ton $ha^{-1}$, while the conventional ones yielded 4.8 ton $ha^{-1}$, representing a significant -21% grain yield. On the contrary, yields for spring crops were not significantly different between the two systems. In general, yields for winter crops at MoLTE were comparable to those in the surrounding areas, while those for spring crops were lower mainly because of the absence of irrigation. Spring crops experienced a significant decrease in yield in both systems, reaching nearly zero production. A factor that probably contributed to this is the introduction of maize in rotation from 2003 to 2009, when rainfall tended to decrease. As maize has a high water requirement, both organic and conventional systems may have suffered from the lack of water; consequently, cultivation of this crop was discontinued. As a matter of fact, some crops like maize that were considered productive under climate conditions in the 1990s were later progressively abandoned in rain-fed systems.

Regarding soil parameters, available $P_2O_5$ decreased over the years both in organic and conventional systems. Since in the organic system $P_2O_5$ fertilizers were almost zero over the span of years, the decrease in available $P_2O_5$ is not linked to organic or conventional management, but probably due to undetermined factors. $P_2O_5$ deficiency may therefore be an additional factor that led to the decrease in yield recorded at MoLTE (2). On the contrary, soil organic matter and total N remained constant over the years in both systems (Table S9). Therefore, the decrease in yields is probably not determined by these two parameters. Differences between the two systems were noted only for organic matter, which showed significantly higher values in the organic system (1.75%) compared to the conventional one (1.6% - Table S9).

### 3.5.2 Energy balance

The impact of organic and conventional practices on energy balance was assessed through the parameters reported in Table S7; results regarding energy inputs ($E$, GJ $ha^{-1}$) are shown in Figure 4.

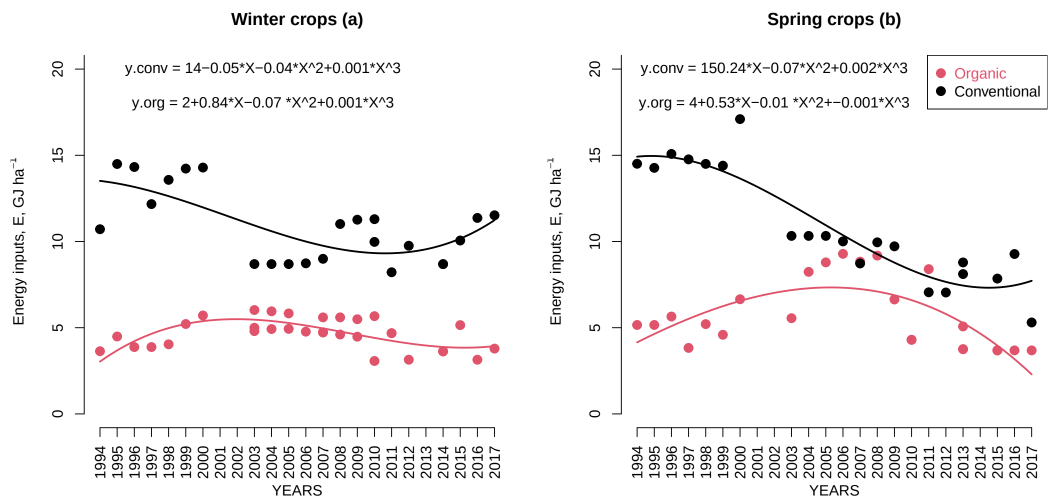


Figure 4: Energy inputs (E) in the organic and conventional systems for winter (a) and spring (b) crops at MoLTE. In the equation, X stands for (YEARS − 1994). Only the crops cultivated simultaneously in both systems were considered.

In the conventional system, an initial marked drop in $E$ was observed both for winter and spring crops, mainly attributed to the reduction of both chemical fertilizers and fuel inputs. This decrease primarily stems from a shift in agronomic practices, transitioning away from the massive use of inputs prevalent in the 1990s to a more restrained use later, mostly due higher a cost of inputs as a cascade effect stemming from increased energy costs. However, while $E$ for winter crops are consistently higher for the conventional system, for spring crops the organic and conventional systems showed comparable $E$, although only between 2004 and 2008. The rise in $E$ in organic systems during those years is due to the green manure introduced to fertilize the subsequent maize crop. This is confirmed by the succeeding decrease of $E$ when green manure was no longer cultivated.

In the face of a growing energy crisis that has resulted in increasing production costs over the years (EC 2023), a comparison between organic and conventional management in terms of $EUE$ may be of considerable relevance (Figure 5). A constant difference can be observed between the two systems in both winter and spring crops. Organic winter crops showed a 33% higher $EUE$ compared to conventional counterparts. Even greater efficiency was observed for spring crops, with a 44% higher $EUE$ in the organic system. Therefore, the organic system undoubtedly performed better in terms of $EUE$ compared to conventional system. This result is consistent with other publications on organic system (Alonso and Guzmán 2010; Ferro et al. 2017; Mäder et al. 2002).

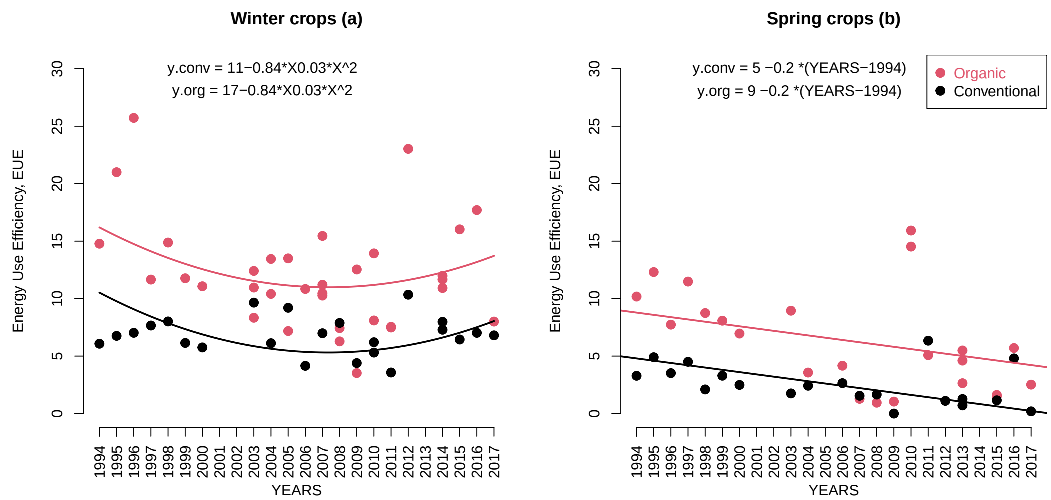


Figure 5: Energy Use Efficiency (EUE) in the organic and conventional systems for winter (a) and spring (b) crops at MoLTE. In the equation, X stands for (YEARS − 1994). Only the crops cultivated simultaneously in both systems were considered.

### 3.5.3 General discussion

From the results found in this study, three main points can be defined:

1. Conventional yield decreases can be attributed to reduced rainfall, a decrease in $P_2O_5$, and reduction in *E*, substantially fertilizers.
2. Organic yield decreases can be solely attributed to reduced rainfall and decrease in $P_2O_5$.
3. The organic system, despite the lower yield, showed a higher *EUE* compared to the conventional system.

Certainly, other factors may have contributed to decreases in yield. Field observations, devoid of supporting data, lead us to speculate that the decrease may also be attributed to the effects of weed competition and crop damage by wild animals. Other factors such as pests and diseases probably had a negligible impact on yield, as their prevalence was not significantly observed in the surveyed area. To sum up, Mediterranean agriculture faces three challenges with regard to the perfect storm:

- to be able to produce enough food for an increasing population;
- to maintain high productivity while consuming less energy;
- to be resilient to water droughts.

The perfect storm concept implies that drivers causing increased food demand and limited availability of energy and water happen simultaneously and that corresponding solutions need to simultaneously address all three crises. Considering the rise in world population and the lower productivity per hectare of organic farming, some authors share the concern that an increase in cultivated area is needed(Tuomisto et al. 2012; Villanueva-Rey et al. 2014). In this context, one possible strategy could be the restoration of a part of the abandoned uncultivated areas in Mediterranean region, which represents one of the areas of the world where processes of land abandonment are widespread (Plieninger et al. 2014). For example in Europe, an estimated 120 Mha of cultivable cropland has been abandoned since 1990 (Levers et al. 2018). This is particularly evident in Italy, where the 21.9 million ha in the 1960s have been reduced nowadays to 12.5 million (ISTAT 2022). Much of this lost agricultural land could easily be returned to production given current availability of technological knowledge, subject to funding research tailored to Mediterranean pedo-climatic and production conditions. Moreover, Italy imports about two-thirds of its energy needs (European Commission 2021). Backed by these considerations and the evidence of long-term dynamics at the MoLTE, we can reasonably state that organic farming is an option to face the perfect storm. Spring crops showed a drastic decrease of productivity in the last 30 years, both under organic and conventional farming in line with the IPCC's worst predictions, due to a decrease of water availability in spring and 1°C increase of temperature. Organically managed soils are more biologically active and less resistant to penetration, which might help farmers store more water and plants reach deeper layers in the soil profile. Such aspects of organic farming are promising but apparently they are not sufficient in coping with water scarcity. This calls for more advanced research on both water stress-resilient crop species and appropriate varieties for organic agriculture. From a climate change adaptation perspective, MoLTE has currently implemented agricultural techniques aimed at enhancing soil organic matter to improve water retention and productivity. These techniques involve the use of organic amendments and a balanced approach to conservation tillage practices, as outlined in the previous study published by Pantani et al. (2022). Moreover, a new crop rotation was introduced in 2019 which includes perennial leguminous species (*Medicago sativa* L.) to counteract the presence of weeds, wheat evolutionary populations (Bocci et al. 2020) and spelt (*Triticum dicoccum* L.) tailored for low-input systems and adapted to semi-arid climate (Table S3). These strategies are designed considering the upward trend of production costs in the near future.

## 3.6 Conclusion

Using a 30-year dataset from the Montepaldi Long-Term Experiment (MoLTE), we showed that rainfed crop yields declined significantly over time in both organic and conventional stockless arable systems. The observed decrease was consistent with a progressive deterioration of the water balance, as indicated by reduced cumulative rainfall during the growing season and increasing maximum temperatures. Therefore, under the Mediterranean conditions represented by MoLTE, water availability emerged as a primary constraint shaping long-term productivity trends, regardless of the management system.

When comparing systems over the period in which the same crops were cultivated simultaneously, organic management maintained a persistent yield penalty for winter cereals ( −21%), whereas spring crops did not differ significantly between organic and conventional systems. Despite this yield gap, the organic system consistently exhibited higher energy use efficiency than the conventional system (+33% for winter crops and +44% for spring crops), reflecting lower energy inputs per unit of output.

Overall, these results indicate that, under Mediterranean rainfed conditions, organic management can improve the energy-efficiency versus yield trade-off, even though it does not fully prevent the long-term decline in yields associated with increasing water limitation. Future work should further clarify the interacting roles of water stress, nutrient dynamics (including the long-term decline in available P), and cropping choices in determining system resilience.

## 3.7 References


Ali E, Cramer W, Carnicer J, Georgopoulou E, Hilmi NJM, Cozannet GL, Lionello P (2022) Mediterranean region. In: Climate change 2022: Impacts, adaptation and vulnerability. Contribution of working group II to the sixth assessment report of the intergovernmental panel on climate change [h.-o. Pörtner, d.c. Roberts, m. Tignor, e.s. Poloczanska, k. Mintenbeck, a. Alegría, m. Craig, s. Langsdorf, s. Löschke, v. Möller, a. Okem, b. Rama (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA Cross-Chapter Paper 4:2233–2272. https://doi.org/10.1017/9781009325844.021

Alonso AM, Guzmán GJ (2010) Comparison of the efficiency and use of energy in organic and conventional farming in spanish agricultural systems. Journal of Sustainable Agriculture 34:312–338. https://doi.org/10.1080/10440041003613362

Altieri MA (1996) Agroecology: The science of sustainable agriculture, second edition (2nd ed.). CRC Press. https://doi.org/https://doi.org/10.1201/9780429495465

Altobelli F, Piazza MG (2022) La gestione sostenibile del suolo: Quali sfide?

Baker P, Mortlock M (2023) Cropgrowdays: Crop growing degree days and agrometeorological calculations

Bird DN, Benabdallah S, Gouda N, Hummel F, Koeberl J, La Jeunesse I, Meyer S, Prettenthaler F, Soddu A, Woess-Gallasch S (2016) Modelling climate change impacts on and adaptation strategies for agriculture in sardinia and tunisia using AquaCrop and value-at-risk. The Science of the total environment 543:1019–1027. https://doi.org/10.1016/j.scitotenv.2015.07.035

Bocci R, Bussi B, Petitti M, et al (2020) Yield, yield stability and farmers' preferences of evolutionary populations of bread wheat: A dynamic solution to climate change. European Journal of Agronomy 121:126156. https://doi.org/https://doi.org/10.1016/j.eja.2020.126156

Bouregaa T (2019) Impact of climate change on yield and water requirement of rainfed crops in the setif region. Management of Environmental Quality: An International Journal 30: https://doi.org/10.1108/MEQ-06-2018-0110

Cammarano D, Ceccarelli S, Grando S, Romagosa I, Benbelkacem A, Akar T, Al-Yassin A, Pecchioni N, Francia E, Ronga D (2019) The impact of climate change on barley yield in the mediterranean basin. European Journal of Agronomy 106:1–11. https://doi.org/https://doi.org/10.1016/j.eja.2019.03.002

Conway GR (1987) The properties of agroecosystems. Agricultural Systems 24:95–117. https://doi.org/https://doi.org/10.1016/0308-521X(87)90056-4

Dowle M, Srinivasan A (2023) Data.table: Extension of ‘data.frame‘

EC (2023) Short-term outlook for EU agricultural markets

Ferro ND, Zanin G, Borin M (2017) Crop yield and energy use in organic and conventional farming: A case study in north-east italy. European Journal of Agronomy 86:37–47. https://doi.org/https://doi.org/10.1016/j.eja.2017.03.002

Fox J, Weisberg S, Price B (2023) Car: Companion to applied regression

Georgopoulou E, Mirasgedis S, Sarafidis Y, Vitaliotou M, Lalas DP, Theloudis I, Giannoulaki KD, Dimopoulos D, Zavras V (2017) Climate change impacts and adaptation options for the greek agriculture in 2021–2050: A monetary assessment. Climate Risk Management 16:164–182. https://doi.org/https://doi.org/10.1016/j.crm.2017.02.002

Gliessman S (2006) Agroecology: The ecology of sustainable food systems, second edition. Agroecology: The Ecology of Sustainable Food Systems, Second Edition 1–389. https://doi.org/10.1201/b17881

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: The challenge of feeding 9 billion people. Science 327:812–818. https://doi.org/10.1126/science.1185383

Hülsbergen K-J, Feil B, Biermann S, Rathke G-W, Kalk W-D, Diepenbrock W (2001) A method of energy balancing in crop production and its application in a long-term fertilizer trial. Agriculture, Ecosystems & Environment 86:303–321. https://doi.org/10.1016/S0167-8809(00)00286-3

IEA (2022) International energy agency (IEA), 2022. World energy outlook 2022. paris. License: CC BY 4.0 (report); CC BY NC SA 4.0 (Annex A):

Iocola I, Bassu S, Farina R, et al (2017) Can conservation tillage mitigate climate change impacts in mediterranean cereal systems? A soil organic carbon assessment using long term experiments. European Journal of Agronomy 90:96–107. https://doi.org/https://doi.org/10.1016/j.eja.2017.07.011

ISTAT (2022) La geografia delle aree interne nel 2020 - vasti territori tra potenzialità e debolezze

Kjeldahl J (1883) A new method for the determination of nitrogen in organic matter. Zeitschrift für Analytische Chemie 22:366–382

Levers C, Schneider M, Prishchepov AV, Estel S, Kuemmerle T (2018) Spatial variation in determinants of agricultural land abandonment in europe. Science of The Total Environment 644:95–111. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.06.326

Lin H-C, Huber J, Gerl G, Hülsbergen K-J (2016) Effects of changing farm management and farm structure on energy balance and energy-use efficiency—a case study of organic and conventional farming systems in southern germany. European Journal of Agronomy 82:

https://doi.org/10.1016/j.eja.2016.06.003

Mäder P, Fliessbach A, Dubois D, Gunst L, Fried P, Niggli U (2002) Soil fertility and biodiversity in organic farming science. Science (New York, NY) 296:1694–7. https://doi.org/10.1126/science.1071148

Marten GG (1988) Productivity, stability, sustainability, equitability and autonomy as properties for agroecosystem assessment. Agricultural Systems 26:291–316. https://doi.org/https://doi.org/10.1016/0308-521X(88)90046-7

Mayer M (2023) Confintr: Confidence intervals

Muller A, Schader C, El-Hage Scialabba N, Brüggemann J, Isensee A, Erb K, Smith P, Klocke P, Leiber F, Stolze M, Niggli U (2017) Strategies for feeding the world more sustainably with organic agriculture. Nature Communications 8:1290. https://doi.org/10.1038/s41467-017-01410-w

Olsen SR, Cole CV, Watanabe FS (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular No 939

Pantani O-L, Ferretti L, Santoni M, Massenzio S, D'Acqui LP, Pacini GC (2022) Assessment of the impact of conventional and organic agroecosystems management options and conservation tillage on soil fertility at the montepaldi long term experiment, tuscany. European Journal of Agronomy 140:126575. https://doi.org/10.1016/j.eja.2022.126575

Plieninger T, Hui C, Gaertner M, Huntsinger L, Webb E (2014) The impact of land abandonment on species richness and abundance in the mediterranean basin: A meta-analysis. PLoS ONE 9:98355. https://doi.org/10.1371/journal.pone.0098355

Potschin-Young M, Haines-Young R (2011) Ecosystem services. Progress in Physical Geography 35:575–594. https://doi.org/10.1177/0309133311423172

Poux X, Aubert PM (2018) An agroecological europe in 2050: Multifunctional agriculture for healthy eating. Findings from the ten years for agroecology (TYFA) modelling exercise. Study N°09/18:

R Core Team (2023) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Ryan JA, Ulrich JM (2023) Xts: eXtensible time series

Saadi S, Todorovic M, Tanasijevic L, Pereira LS, Pizzigalli C, Lionello P (2015) Climate change and mediterranean agriculture: Impacts on winter wheat and tomato crop evapotranspiration, irrigation requirements and yield. Agricultural Water Management 147:103–115. https://doi.org/https://doi.org/10.1016/j.agwat.2014.05.008

Sarkar D (2008) Lattice: Multivariate data visualization with R

Sarkar D (2023) Lattice: Trellis graphics for r

Sax C (2023) Tsbox: Class-agnostic time series

Spinu V, Grolemund G, Wickham H (2023) Lubridate: Make dealing with dates a little easier

Tuomisto HL, Hodge ID, Riordan P, D. W. M (2012) Does organic farming reduce environmental impacts?–a meta-analysis of european research. J Environ Manage 112:309-20:Epub 2012 Sep 1. PMID: 22947228. https://doi.org/doi: 10.1016/j.jenvman.2012.08.018

Villanueva-Rey P, Vázquez-Rowe I, Moreira MT, Feijoo G (2014) Comparative life cycle assessment in the wine sector: Biodynamic vs. Conventional viticulture activities in NW spain. Journal of Cleaner Production 65:330–341. https://doi.org/https://doi.org/10.1016/j.jclepro.2013.08.026

Waha K, Krummenauer L, Adams S, et al (2017) Climate change impacts in the middle east and northern africa (MENA) region and their implications for vulnerable population groups. Regional Environmental Change 17: https://doi.org/10.1007/s10113-017-1144-2

Walkley AJ, Black IA (1934) Estimation of soil organic carbon by the chromic acid titration method. Soil Sci 37:29–38

Wickham H (2023) Tidyverse: Easily install and load the tidyverse

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Writing – review & editing: Margherita Santoni, Ottorino-Luca Pantani, Francesco Serafini, Lorenzo Ferretti, Jean-Francois Vian, Gaio Cesare Pacini

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- FertilCrop: Fertility Building Management Measures in Organic Cropping Systems (CORE Organic Plus Funding Bodies, H2020 ERA-Net, 2015- 2018, Grant Agreement no. 618107).

## 3.11 Supplementary Materials

Table S1: Main soil physico-chemical characteristics at MoLTE in 1992.

| Parameter | Organic | Conventional |
|---|---|---|
| Gravel (%) | 6.3 | 6.1 |
| Sand (%) | 20.2 | 21.0 |
| Silt (%) | 46.3 | 44.6 |
| Clay (%) | 32.9 | 33.8 |
| pH ($H_2O$) | 8.3 | 8.3 |
| CEC ($cmol^+ kg^{-1}$) | 17.6 | 19.4 |
| Organic matter (%) | 1.70 | 1.67 |
| Total N ($g\ kg^{-1}$) | 1.06 | 1.09 |
| Total $P_2O_5$ ($mg\ kg^{-1}$) | 1633.5 | 1600.0 |
| Available $P_2O_5$ ($mg\ kg^{-1}$) | 22.8 | 29.6 |
| Exchangeable $K_2O$ ($mg\ kg^{-1}$) | 171.8 | 134.5 |

Table S2: Ordinary tillage operations, fertilization and weeding at MoLTE. During the 30 years of the MoLTE experiment, ordinary agronomic operations could have changed due to year-specific production and climatic conditions.

| | Winter crops | | Spring crops | |
|---|---|---|---|---|
| Operation | Organic | Conventional | Organic. | Conventional. |
| Primary tillage | Plowing or chisel plowing | Plowing or chisel plowing | Plowing or chisel plowing | Plowing or chisel plowing |
| Pre-sowing fertilization | Green manure or organic fertilizer | Diammonium phosphate | Green manure or organic fertilizer | NPK: 20.10.10 |
| First fertilization | – | Ammonium nitrate | – | Ammonium nitrate |
| Second fertilization | – | Urea | – | Urea |
| Chemical weeding | – | Herbicides | – | Herbicides |
| Mechanical weeding | Weed harrowing | Weed harrowing | Weed hoeing | Weed hoeing |
| Disease control | – | – | – | – |
| Insect control | – | – | – | – |

Table S3: Crop rotations from 1992 to 2022 at MoLTE.

| Period | Rot. | Organic | Conventional |
| --- | --- | --- | --- |
| 1992–2000 | 1. | Sunflower | Sunflower |
| | 2. | Faba bean | Winter common wheat / winter barley |
| | 3. | Winter common wheat / winter barley | |
| | 4. | Clover | |
| 2000–2004 | 1. | Green manure / maize | Maize |
| | 2. | Faba bean | Winter barley |
| | 3. | Winter barley | |
| | 4. | Clover | |
| 2004–2008 | 1. | Green manure / maize | Maize |
| | 2. | Faba bean | Winter durum wheat / winter common wheat |
| | 3. | Winter durum wheat / winter common wheat | |
| | 4. | Clover | |
| 2008–2013 | 1. | Sunflower | Sunflower |
| | 2. | Faba bean | Winter durum wheat / winter common wheat |
| | 3. | Winter durum wheat / winter common wheat | |
| | 4. | Alfalfa | |
| 2013–2019 | 1. | Sunflower / green manure + millet | Sunflower |
| | 2. | Chickpea / lentil | Winter barley |
| | 3. | Winter barley / ancient winter common wheat | |
| | 4. | Clover | |
| 2019–2022 | 1. | Ancient winter common wheat | Alfalfa |
| | 2. | Spelt | Alfalfa |
| | 3. | Alfalfa | |
| | 4. | Alfalfa | |

Table S4: Crops under analysis, crop acronyms and number of yield observations in organic (Obs. Org.) and conventional (Obs. Conv.) from 1994 to 2017 at MoLTE. Only data where crops were cultivated simultaneously in organic and conventional system were chosen.

| Winter crops | | | | Spring crops | | | |
|---|---|---|---|---|---|---|---|
| Species | Acronym | Obs. Org. | Obs. Conv. | Species | Acronym | Obs. Org. | Obs. Conv. |
| *Hordeum vulgare* L. | BA | 14 | 9 | *Zea mays* L. | MA | 7 | 7 |
| *Triticum aestivum* L. | WC | 10 | 9 | *Helianthus annuus* L. | SU | 16 | 13 |
| *Triticum durum* L. | WD | 10 | 5 | | | | |

Table S5: Linear models for the average yields (t $ha^{-1}$) for each crop. In the equation, CONV = 1 and ORG assumes the values 0 and 1 when the management is Conventional or Organic, respectively; YEAR is 0, 1, 2, ..., 24 where 0 is the year 1994. The coefficients are reported when the significance was $< 0.05$, otherwise ”ns” is reported.

| Crop species | Equation |
|---|---|
| Winter barley (BA) | $3.81 \times \text{CONV} - 0.92 \times \text{ORG}$ |
| Winter common wheat (WC) | $2.70 \times \text{CONV} - 0.42 \times \text{ORG}$ |
| Winter durum wheat (WD) | ns |
| Maize (MA) | $-0.29 \times \text{YEAR}$ |
| Sunflower (SU) | $-0.07 \times \text{YEAR}$ |

Table S6: Number of observations for the main soil chemical analyses from 1994 to 2017 in organic and conventional systems at MoLTE.

| Parameter | Organic | Conventional |
|---|---|---|
| Organic matter (%) | 35 | 22 |
| Total N (g $kg^{-1}$) | 35 | 23 |
| Available $P_2O_5$ (mg $kg^{-1}$) | 32 | 21 |
| Exchangeable $K_2O$ (mg $kg^{-1}$) | 29 | 18 |

Table S7: Definitions of energy parameters.

| Energy parameter | Definition | Unit |
|---|---|---|
| Direct energy input (Ed) | Input of diesel | GJ $ha^{-1}$ $y^{-1}$ |
| Indirect energy input (Ei) | Seed + mineral and organic fertilizers + herbicides + machines | GJ $ha^{-1}$ $y^{-1}$ |
| Energy output (Eo) | Energy in the harvested biomass | GJ $ha^{-1}$ $y^{-1}$ |
| Energy input (E) | E = Ed + Ei | GJ $ha^{-1}$ $y^{-1}$ |
| Energy Use Efficiency (EUE) | EUE = Eo/E | dimensionless |

Table S8: Energy equivalents used for inputs and outputs at MoLTE.

| Item | Energy equivalents (MJ $unit^{-1}$) | References |
|---|---|---|
| **Machinery operations** | | |
| Diesel fuel (l) | 39.6 | Hülsbergen et al. 2001 |
| Machines (kg) | Depending on soil tillage operations | Data collected at MoLTE |
| **Mineral and organic fertilizers (kg)** | | |
| N | 35.3 | Hülsbergen et al. 2001 |
| $P_2O_5$ | 15.8 | Hülsbergen et al. 2001 |
| Manure | 0.3 | Dal Ferro et al. 2017 |
| **Herbicides (kg)** | | |
| Herbicides (kg) | 288 | Hülsbergen et al. 2001 |
| **Seed (kg)** | | |
| Winter wheat | 5.5 | Hülsbergen et al. 2001 |
| Winter barley | 5.5 | Hülsbergen et al. 2001 |
| Sunflower | 12 | Lin et al. 2016 |
| Maize | 14.6 | Simon et al. 2021 |
| **Grain yield output (kg)** | | |
| Winter wheat | 18.6 | Hülsbergen et al. 2001; Migliorini et al. 2012 |
| Winter barley | 18.6 | Hülsbergen et al. 2001; Migliorini et al. 2012 |
| Sunflower | 26.8 | Lin et al. 2016 |
| Maize | 14.7 | Dal Ferro et al. 2017; Migliorini et al. 2012 |

Table S9: Linear models for the considered soil parameters. In the equation, CONV = 1 and ORG assumes the values 0 and 1 when the management is Conventional or Organic, respectively; YEAR is 0, 1, 2, ..., 24 where 0 is the year 1994. The coefficients are reported when the significance was $< 0.05$, otherwise "ns" is reported.

| Soil parameter | Equation |
|---|---|
| Organic matter (%) | $1.60 \times \text{CONV} + 0.15 \times \text{ORG}$ |
| Total N (g $kg^{-1}$) | ns |
| Available $P_2O_5$ (mg $kg^{-1}$) | $-25.8 \times \text{YEAR} + 0.65 \times \text{YEAR}^2$ |

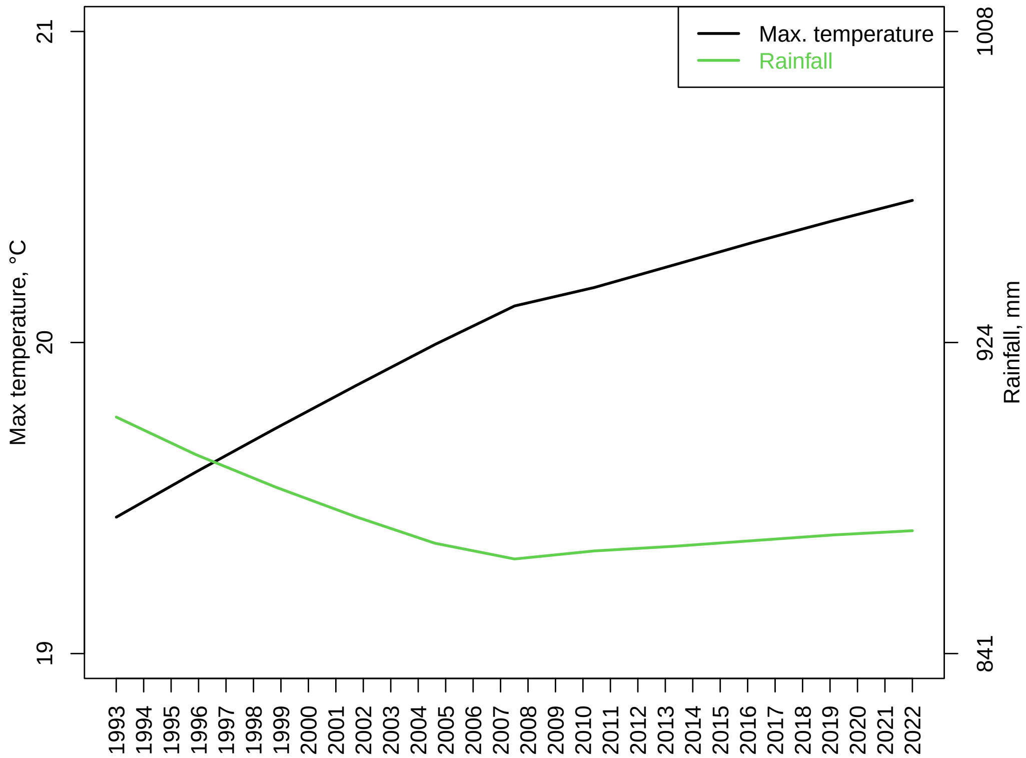


Figure S1: Trends in maximum temperature (°C) and rainfall (mm) from 1993 to 2022, obtained from MoLTE data and processed using 'stl' function. An increase in maximum temperatures by 1°C and a decrease in rainfall by -166 mm were recorded.

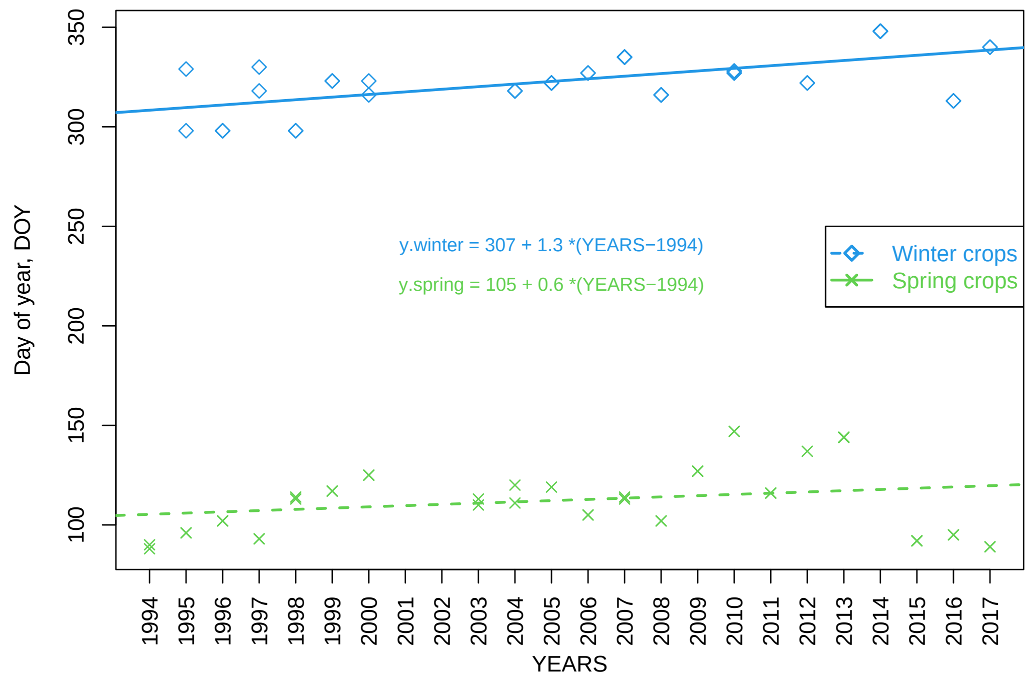


Figure S2: Sowing dates for winter and spring crops at MoLTE. Only the crops cultivated simultaneously in organic and conventional systems were considered. Dotted line represents no significant difference in sowing dates (p 0.05).

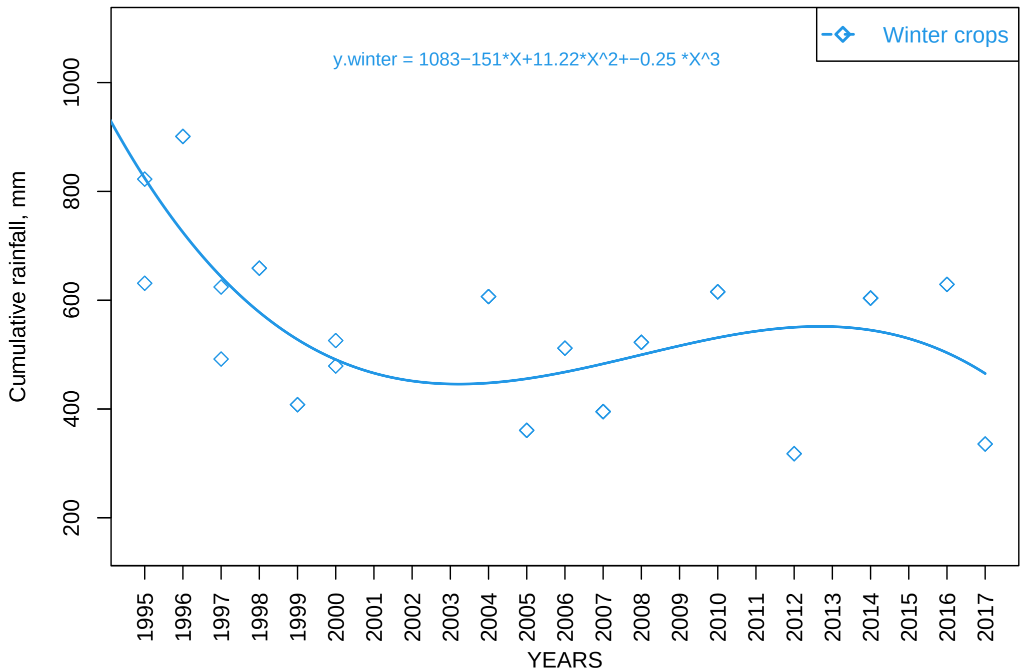


Figure S3: Cumulative rainfall (mm) during the vegetative cycle of winter crops, keeping constant the sowing date, i.e. the earliest sowing dates recorded at MoLTE throughout the years, which was October $25^{th}$. In the equation, X stands for (YEARS − 1994). Only the crops cultivated simultaneously in organic and conventional systems were considered.

# 4 A Systematic Review of Scientific Research on Municipal Solid Waste Compost: Impacts on Crop Productivity and Soil Fertility

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## 4.1 Abstract

Purpose

The increasing need for the disposal of Municipal Solid Waste (MSW) and the decline in soil organic matter in cultivated soils represent two major challenges that could be addressed through the recycling of the organic fraction of MSW into compost (MSWC) for agricultural use. This review assesses the agronomic effects of MSWC on crop productivity and soil fertility, highlighting key findings, limitations, uncertainties and research needs.

Methods

The selection from Web Of Science gave 115 papers (1985-March 2025) and was performed by Latent Dirichlet Allocation (LDA) topic modeling, focusing the search on the effects of MSWC on crop productivity across arable, horticultural and orchards production systems, as well as its impact on soil physical, chemical, and biological properties. Only peer-reviewed articles in English were included, and selection was restricted to studies reporting original quantitative data on productivity or soil fertility. No formal risk of bias assessment was conducted.

Results

The papers are distributed among Europe (43%), Asia (31%), North America (11%), Africa (7%), South America (6%) and Oceania (2%). Application rates varied widely (5-875 t ha$^{-1}$), with consistently positive effects generally reported at medium-high doses (>30-40 t ha$^{-1}$). On average, yield increases were +63.3% in arable systems, +37.3% in horticultural crops and +23% in orchards compared to unamended controls, while soil organic matter rose by up to +2.5% under field conditions and +248% in pot experiments. Particularly strong benefits were observed in degraded or saline-sodic soils, where MSWC reduced soil EC by up to -39% and increased SOM by up to +45%. However, most studies were short-term (only 18% >5 years), and many lacked detailed compost characterization, limiting evidence on long-term nutrient cycling, carbon sequestration and contaminant dynamics.

Conclusions

Evidence synthesized in this review suggests that MSWC application may support circular waste management strategies and reduce reliance on mineral fertilizers in certain cropping systems, particularly when applied at medium to high rates and under suitable pedoclimatic conditions. Nevertheless some challenges remain related to its variable composition, the need for high application rates, and risks associated with contaminants such as heavy metals, mi-

croplastics and other contaminants. Further long-term studies are needed to optimize application strategies and assess its sustainability in agricultural systems.

## 4.2 Keywords

Urban waste management; Soil health; Crop performance; Circularity; Sustainable agriculture

## 4.3 Introduction

From time immemorial, organic wastes were used to enhance and maintain soil fertility and support agricultural productivity, fostering a natural nutrient cycle (Barles 2014; Güldner and Krausmann 2017). The industrialization of agriculture and the widespread adoption of mineral fertilizers during the 20th century reduced the amount of organic inputs almost to zero (Manlay et al. 2007). Agricultural intensification and specialization resulted in a concentration of livestock farms in limited areas, associated with the accumulation of organic residues, while areas with low livestock density face a deficit in soil organic matter (SOM), crucial for maintaining soil fertility and crop productivity (Feller et al. 2012; Garrett et al. 2020).

The rapid growth of the urban population has significantly increased the amounts of municipal solid waste (MSW), which currently exceed 2.0 billion tonnes $yr^{-1}$ globally and are projected to increase by approximately 70% by 2050, reaching about 3.4 billion tonnes. Waste collection efficiency varies considerably according to Gross Domestic Product (GDP) per capita, with high-income countries collecting over 90% of generated waste, while low-income countries collect less than 40%. Only 19% of global MSW is currently recycled, representing a missed opportunity to recover agronomically valuable organic matter (OM) (Kaza et al. 2018). Organic material constitutes a substantial proportion of MSW, yet 38% of global MSW remains unmanaged, either dumped or openly burned, exacerbating environmental degradation through greenhouse gas emissions and soil and water contamination (Chen et al. 2020; United Nations Environment Programme and International Solid Waste Association 2024). Landfilling and incineration, the traditional disposal methods, further contribute to these environmental impacts, underscoring the urgency of sustainable waste management strategies (Lou and Nair 2009). Circular waste management approaches, which prioritize the recovery and reuse of the organic fraction of MSW, align with the FAO's ten Elements of Agroecology, particularly with the principles of recycling and with circular and solidarity economy. Recycling OM, rather than burning it, helps to locally close nutrient cycles, to reduce reliance on external inputs, and to enhance resilience to market and climate variability. Repurposing OM and by-products not only supports sustainable agricultural practices, but also fosters agroecological innovation, offering new opportunities for resource-efficient farming systems (FAO 2018).

One of the most effective strategies for managing the organic fraction of MSW is composting (Babu et al. 2021). It transforms organic waste into a stable and nutrient-rich material through microbial activity, reducing waste volume and producing a valuable resource for agricultural use. Composting ensures that wastes can be safely reintegrated into farming systems by stabilizing OM and eliminating pathogens (Misra et al. 2003).

Municipal Solid Waste Compost (MSWC) is increasingly recognized as a valuable resource for agriculture. When distributed in the field, MSWC improves soil structure, increases SOM content, and provides essential nutrients like N, P, and K, both for crops and microbial community(Diacono and Montemurro 2010; Hargreaves et al. 2008). It is therefore most beneficial for degraded soils and low-input farming systems. Additionally, MSWC aligns with the principles of the circular economy by returning OM to the soil, reducing reliance on mineral fertilizers, and minimizing the environmental footprint of waste management (Razza et al. 2018; Valverde-Orozco et al. 2024). Although MSWC composition depends on feedstock and processing, variability is greater across producers and compost types than within a given producer; this pattern directly affects reproducibility and agronomic performance (Manea et al. 2024). While national legislation and producer compliance typically limit regulated contaminants in MSWC to legally acceptable levels, uncertainties persist for emerging contaminants (e.g., microplastics) and in less-regulated contexts, representing a potential risk to soil quality and crop safety; therefore, advanced processing technologies and strict quality controls are required to mitigate these drawbacks and ensure the safe agronomic use of MSWC (Belén Almendro-Candel et al. 2019).

The agronomic effectiveness of MSWC is also highly dependent on environmental and agronomic contexts. Its benefits vary with crop type, soil properties, and application rates, often requiring high doses to achieve rapid and significant results (Cao et al. 2023). While effective at improving soil health and productivity, such high doses may not always be practical or economically feasible for farmers. Furthermore, there is a limited understanding of the cumulative impacts of MSWC over time, including its effects on soil C sequestration, microbial communities, and nutrient cycling.

The agronomic utilization of MSWC provides a pathway for recycling MSW, but questions remain about its potential to mitigate greenhouse gas emissions and reduce reliance on mineral fertilizers. Few studies have examined its role in large-scale agricultural systems or its integration into circular waste management models. Addressing the limited understanding of its agronomic performance at field scale and its role in resource-efficient waste valorization, requires multidisciplinary research efforts. This includes long-term field experiments, advancements in processing technologies to improve compost quality and consistency, and comprehensive risk assessments to evaluate potential environmental and economic trade-offs. Such research efforts are essential to optimize MSWC production and application while ensuring environmental and economic sustainability along the entire chain.

Several reviews have investigated the role of organic amendments in agriculture (Diacono and

Montemurro 2010; Hargreaves et al. 2008; Martínez-Blanco et al. 2013), but none have specifically and systematically evaluated the agronomic effects of Municipal Solid Waste Compost (MSWC). The aim of this review is therefore to provide an updated and in-depth synthesis of MSWC application in agricultural systems, with a focus on crop productivity and soil fertility outcomes derived from both field and pot experiments. The underlying hypothesis is that MSWC, owing to its organic matter content and nutrient supply, can positively influence soil physical, chemical, and biological properties, ultimately supporting crop production. By summarizing current knowledge, highlighting variability across crops, soils, and climatic conditions, and identifying knowledge gaps, this review seeks to advance sustainable waste management practices and improve the integration of MSWC into agricultural systems.

## 4.4 Material and Methods

### 4.4.1 Review design, information sources and search strategy

This study adopted a structured literature review approach, supported by computational techniques for literature screening and topic refinement. The review focused on peer-reviewed scientific articles published in high-impact journals and indexed in the Web of Science™ (WoS) Core Collection between January 1985 and March 2025.

The review followed the PRISMA 2020 guidelines (Page et al. 2021) for systematic reviews to ensure transparency and reproducibility. A schematic overview of the article selection process, adapted from the PRISMA 2020 flow diagram, is provided Fig. 6. The diagram summarizes each step of the screening and refinement process, from the initial WoS query to the final manual selection of 115 articles specifically addressing the use of MSWC in agriculture.

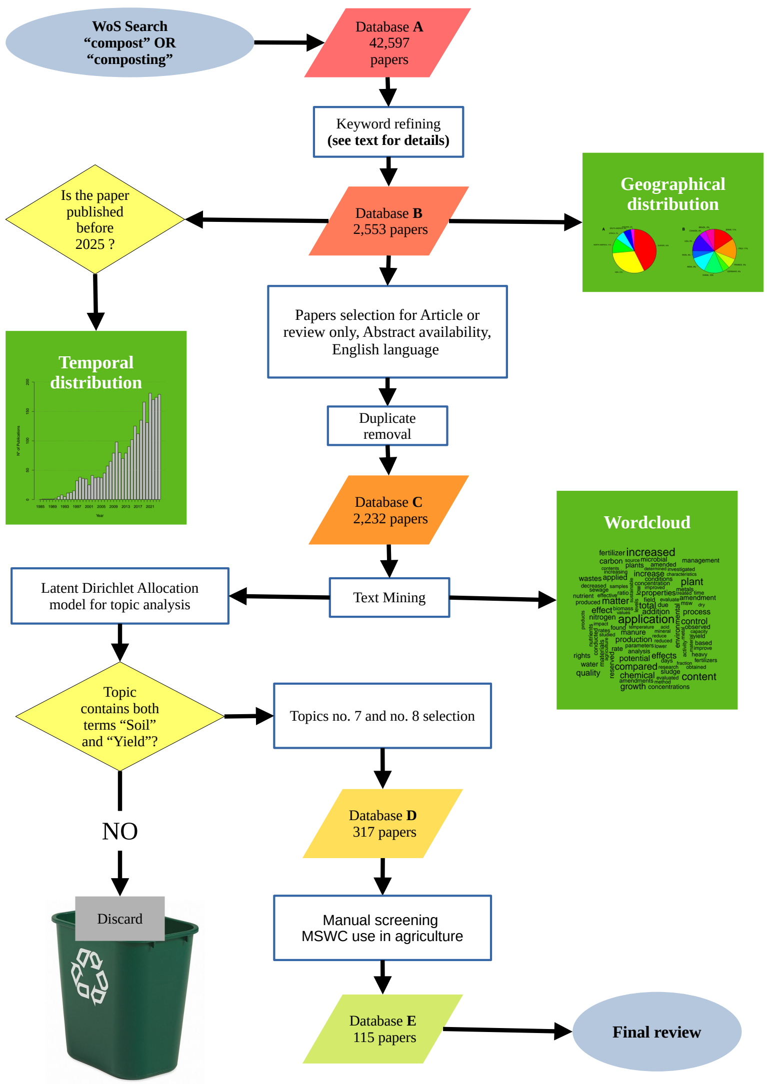


Figure 6: PRISMA 2020 flow diagram of the literature screening. From the initial Web of Science (WoS) query (n = 42,597) to keyword-refined records (n = 2,553), then LDA topic modeling (n = 2,232) and selection of “yield” topics (n = 317), culminating in the final corpus (n = 115) after manual screening. Abbreviations: PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses; LDA = Latent Dirichlet Allocation; MSWC = municipal solid-waste compost.

The initial search in the WoS database was conducted using the keywords “compost” OR “composting”, which yielded a set of 42,597 papers (database A, Fig. 6).

Some additional keywords such as “organic waste” OR “municipal solid waste” OR “urban food waste” OR “urban solid biowaste” OR “urban waste” OR “urban biowaste” were then added to further refine the search and focus on the application of MSWC in agriculture The search was furtherly narrowed down by including the terms “soil fertility” OR “soil” OR “crop productivity” to specifically target articles that examined the impact of MSWC on crop productivity and soil fertility. This process yielded 2,553 paper (database B, Fig. 6). The final search was performed on March 18, 2025.

Database B was analysed to assess both temporal and spatial trends, providing insights into the evolution of research activity over time and the geographical distribution of studies. For the temporal analysis, papers from 2025 were excluded to avoid partial data that would not accurately represent the total research output for the year.

Database C (2,232 articles, Fig. 6) was generated by database B by: i) selecting “Article” and “Review” categories with abstracts available; ii) selecting English peer-reviewed articles; iii) removing duplicates.

### 4.4.2 Text mining and topic refinement

Text Mining approach (Welbers et al. 2017), which analyzes individual words in the abstracts by examining their frequency to extract meaningful patterns from data, was then applied to the papers included in Database C. The core text mining procedures were carried out using the R package *tidytext* (Silge and Robinson 2016). The preprocessing steps included the removal of common stop words (e.g., “and,” “the”, “of”) to retain only the most informative terms.

Subsequently, the text from the abstracts was tokenized into individual words, allowing for the transformation of the database into a structured format suitable for analysis. Following the text mining process, the tokenized output was used to generate a word cloud using the *wordcloud* package in R (Fellows et al. 2018), displaying the 100 most frequent terms in the database. To improve the representativeness of the visualization, some obvious words -e.g. “soils”, “composts”, “composted” “study”, “experiment”, “results”, “significant”, “significantly”, “treatment”, ”treatments” - were excluded from the word cloud.

The Latent Dirichlet Allocation model (LDA), a widely recognized algorithm for topic modelling, was used to refine the selection. This model is an unsupervised machine learning algorithm that identifies underlying topics within a document corpus by modelling the probabilistic distribution of words across documents (Jelodar et al. 2019). The LDA model provides a more structured examination of the research field by organizing the literature into distinct thematic clusters and it was fitted using the *topicmodels* package in R (Grün and Hornik 2011).

Each topic is characterized by two key outputs of the LDA model, i.e., $\beta$ and $\gamma$. The first output, $\beta_{k,w}$, represents the probability of term $w$ belonging to topic $k$. It is computed using the proportion of term occurrences within a topic relative to the overall term distribution across all topics and it is calculated as:

$$\beta_{k,w} = \frac{\sum_{d=1}^{D} \sum_{n=1}^{N} \phi_{d,n,k} \cdot \mathbf{1}(w_{d,n} = w)}{\sum_{w'} \sum_{d=1}^{D} \sum_{n=1}^{N} \phi_{d,n,k} \cdot \mathbf{1}(w_{d,n} = w')}$$

where:

- $D$ is the total number of documents in the database,
- $N$ is the number of words in document $d$,
- $\phi_{d,n,k}$ is the posterior probability that word $n$ in document $d$ is generated by topic $k$,
- $w_{d,n}$ is the $n$-th word in document $d$,
- $w'$ denotes any term in the vocabulary,
- $\mathbf{1}()$ is an indicator function, equal to 1 if the condition is true, 0 otherwise.

The second output, $\gamma_{d,k}$, indicates the proportion of topic $k$ in a specific document $d$, was updated iteratively as:

$$\gamma_{d,k} = \alpha_k + \sum_{n=1}^{N} \phi_{d,n,k}$$

where:

- $N$ is the number of words in document $d$,
- $\phi_{d,n,k}$ is the posterior probability that the $n$-th wordin document $d$ is assigned to topic $k$,
- $\alpha_k$ is the Dirichlet prior for topic $k$.

In other words, $\beta$ emphasizes the word distribution within topics and $\gamma$ reflects the topic distribution within documents.

After fitting the LDA model, each document was assigned to the topic with the highest posterior probability. This was achieved by grouping the documents based on the $\gamma_{d,k}$ values, which indicate the relative contribution of each topic to the content of a document. Applying this approach, 15 thematic clusters were identified. Among these, topics no. 7 and no. 8 were selected for further analysis because they were the only clusters in which the keyword "yield" appeared, in addition to "soil". Since soil was present across nearly all topics, reflecting its inclusion in the original WoS search string, yield was considered the key discriminating factor to identify studies explicitly linking the two core outcomes of this review, namely crop productivity and soil fertility. This intentionally restrictive criterion allowed us to focus on the corpus most relevant to the objectives of the review, resulting in database D with 317 papers (Fig. 6).

### 4.4.3 Study selection and screening process

The relevance of all 317 articles in Database D was assessed through full-text screening. Studies were included if they: i) reported original quantitative data on crop productivity and/or soil fertility; ii) focused on the application of MSWC (and not other organic amendments) and iii) presented field or pot experiments under agricultural conditions.

Title and abstract screening was conducted by one reviewer, while full-text assessment was independently performed by two reviewers. Disagreements were resolved by discussion and consensus. No automation tools were used for study selection.

After applying these criteria, 115 articles were selected and included in the final dataset (Database E, Fig. 6).

The studies included were then classified according to their main objective, trial duration, production system, experimental methods, and study conditions.

Although no formal review protocol was registered, the entire selection process was designed in accordance with the PRISMA 2020 statement. The final number of included studies and reasons for exclusion are documented in the flow diagram (Fig. 6), following the four standard phases: identification, screening, eligibility, and inclusion.

### 4.4.4 Data extraction and synthesis strategy

Quantitative data on MSWC application rates (t $ha^{-1}$ $yr^{-1}$, dry matter basis), variation in crop productivity (%), and soil organic matter (SOM, %) content for both treated and untreated plots were extracted when reported. Values were obtained directly or, when necessary, calculated from published figures and tables. Nutrient loads (kg $ha^{-1}$ $yr^{-1}$ of total N, P, K) were derived by combining reported MSWC composition (% d.m.) with the average annual application rate (see Tables S10–S11). Unless otherwise stated in the original articles, yield and soil responses are interpreted relative to unamended controls. When studies explicitly used different baselines (e.g., compost-only baseline or mineral-fertilized controls), this is indicated in the text. No meta-analysis was performed due to the high heterogeneity in experimental conditions, study designs, compost compositions, and reported outcome metrics. Instead, we conducted a narrative synthesis without subgroup or sensitivity analyses. To contextualize site conditions, we summarized the distribution of soil texture classes across experimental sites (Table S12), and we documented data coverage and ranges for compost composition (Table S10), field nutrient loads (Table S11), and contaminants (Tables S13–S15).

The papers selected in this review therefore outline a landscape shaped by interactions between MSWC and multiple agronomic and environmental factors. We examine this landscape from two complementary "windows": one centered on crops and yield, the other on soil fertility. From the first, we track outcomes across cereals, vegetables, and trees; from the second, we assess the physical, chemical, and biological dimensions of soil fertility. This analytical separation is purely heuristic: crop productivity and soil fertility are tightly coupled, and observed outcomes are the result of numerous, interacting factors operating across scales.

### 4.4.5 Data availability and supplementary material

The full list of reviewed articles included in database E is provided in the Supplementary Material. The file "reviewed_papers.csv"in Supplementary Material includes data on the average annual MSWC application rate, the mean variation in crop productivity, and the average values of SOM for both untreated plots and those treated with MSWC. Details on how these values were calculated are also provided in the file description in Supplementary Material. These materials, along with the R scripts used for text mining and topic modeling, can be made available upon request. In addition, the Supplementary Material contains several tables and figures supporting data interpretation and transparency.

## 4.5 Results

### 4.5.1 Study selection

A total of 115 studies met the inclusion criteria for this review (Fig. 6). These articles specifically addressed the use of MSWC in agriculture and reported quantitative data on crop productivity or soil fertility. While some initially relevant studies were excluded - for example, those lacking quantitative results, focusing on other organic waste types, or consisting of review articles without original data - the final dataset represents a consistent and robust evidence base. Full details of the screening workflow and quality assessment criteria are reported in the Materials and Methods section, and the complete list of included studies is provided in the Supplementary Materials ("reviewed_papers.csv").

### 4.5.2 Geographical and temporal distribution

The geographical and temporal trends presented in this section (Fig. 7 and Fig. 8) result from the analysis of database B (Fig. 6) identified through the structured literature survey.

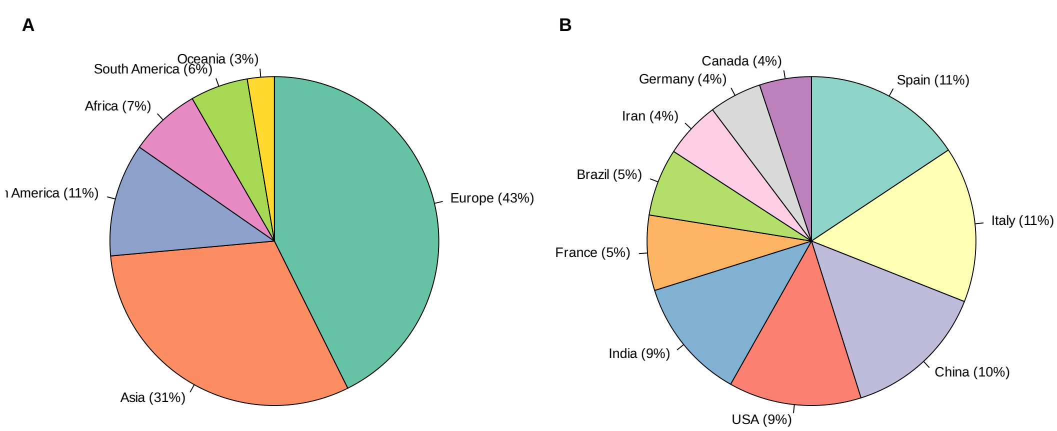


Figure 7: Global distribution of articles (1985–2024) included in this review (n = 115) from the Web of Science (WoS) Core Collection. **A**: Share of studies by continent. **B**: Top ten countries by publication share. Percentages are computed over all records; studies conducted in multiple countries contribute to each listed country, so panel totals sum to 100%.

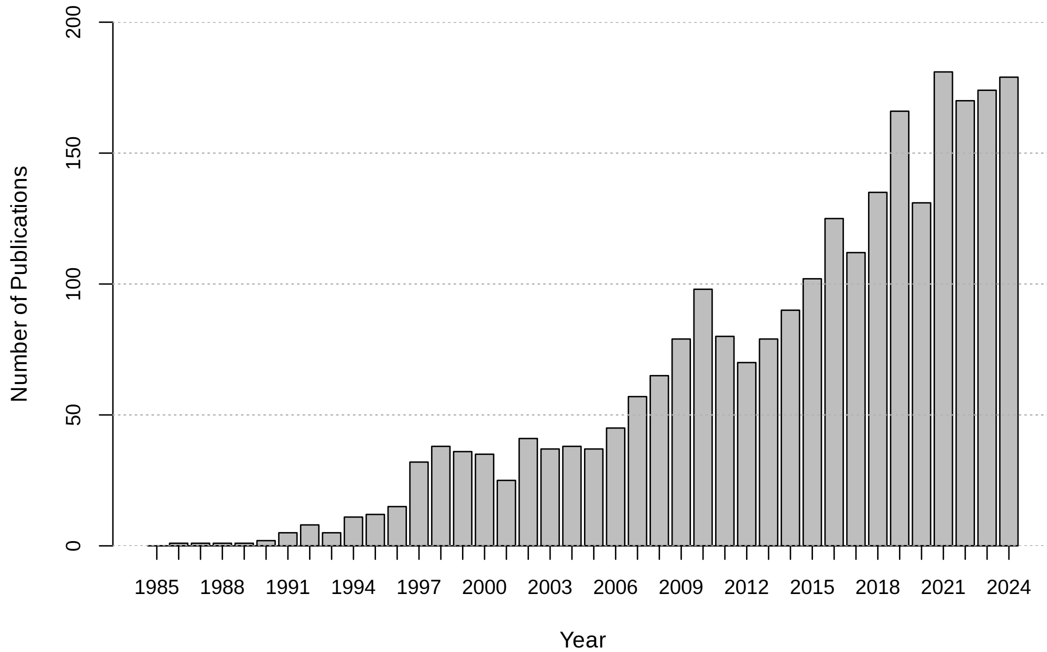


Figure 8: Annual number of publications (1985–2024) on MSWC in agriculture retrieved from the Web of Science (WoS) Core Collection (n = 115). Counts refer to peer-reviewed journal articles included in the final database of this review. Abbreviation: MSWC = municipal solid-waste compost.

The global distribution of peer-reviewed articles published between 1985 and 2024[3], as indexed in the WoS Core Collection, is illustrated in Fig. 7. As shown in panel A, Europe emerges as the leading contributor, accounting for 43% of the total publications, and it is followed by Asia (31%) and North America (11%). Africa (7%) and South America (6%). Oceania represents the smallest share, with 2% of the total.

Panel B provides a closer look at the top 10 countries by publication share. Spain and Italy are the most prolific, each contributing to 11% of the total number of articles. China and India also rank highly, with 10% and 9%, respectively. Other notable contributors include France (5%), Brazil (5%), United States (9%), Canada (4%), Germany (4%), and Iran (4%).

Fig. 8 shows the number of articles on MSWC from 1985 to 2024. The number of publications has steadily increased, particularly from the late 1990s onward, indicating a growing recognition

[3]Being the last query on WoS done on March 2025, this year is considered incomplete and therefore not included in the temporal analysis

of the importance of this research domain. Initially, there were very few publications, with less than 10 articles per year, reflecting the nascent stage of research into composting and its agricultural applications. However, the early 2000s marked a substantial rise in research output, corresponding with heightened global awareness of environmental sustainability and the introduction of policies aimed at reducing waste through recycling and composting. The number of publications continued to grow, peaking in 2021 with 181 articles, which accounted for 7.49% of the total publications. While the number declined slightly to 170 in 2022, it reached 174 in 2023 and 179 in 2024, suggesting a sustained high level of research activity in the field.

### 4.5.3 Text Mining and Topic Analysis

A Text Mining analysis was performed on database C (Fig. 6) to provide a descriptive overview of recurrent terminology in the abstracts and to support the subsequent refinement of the literature corpus. After standard preprocessing (lowercasing, removal of numeric-only tokens, short words, and stopwords, including search-string and generic terms), a word cloud representing the 100 most frequent terms was generated (Fig. 9). This representation highlights the frequent occurrence of terms such as “increased” and “increase”, reflecting the widespread reporting of positive effects of MSWC on various agronomic and soil parameters. The term “matter” also stands out, most likely in reference to OM or SOM. The presence of “rate” and “rates” suggests a consistent focus on application strategies and their impact on both crop performance and soil response. Additionally, although less prominent, the appearance of “microbial”, “heavy”, “metal”, and “metals” confirms that both biological activity and potential contamination are recurring investigated concerns. Notably, other forms of contamination such as microplastics do not emerge among the 100 most frequent terms, suggesting that this issue remains largely unexplored in the current body of research.

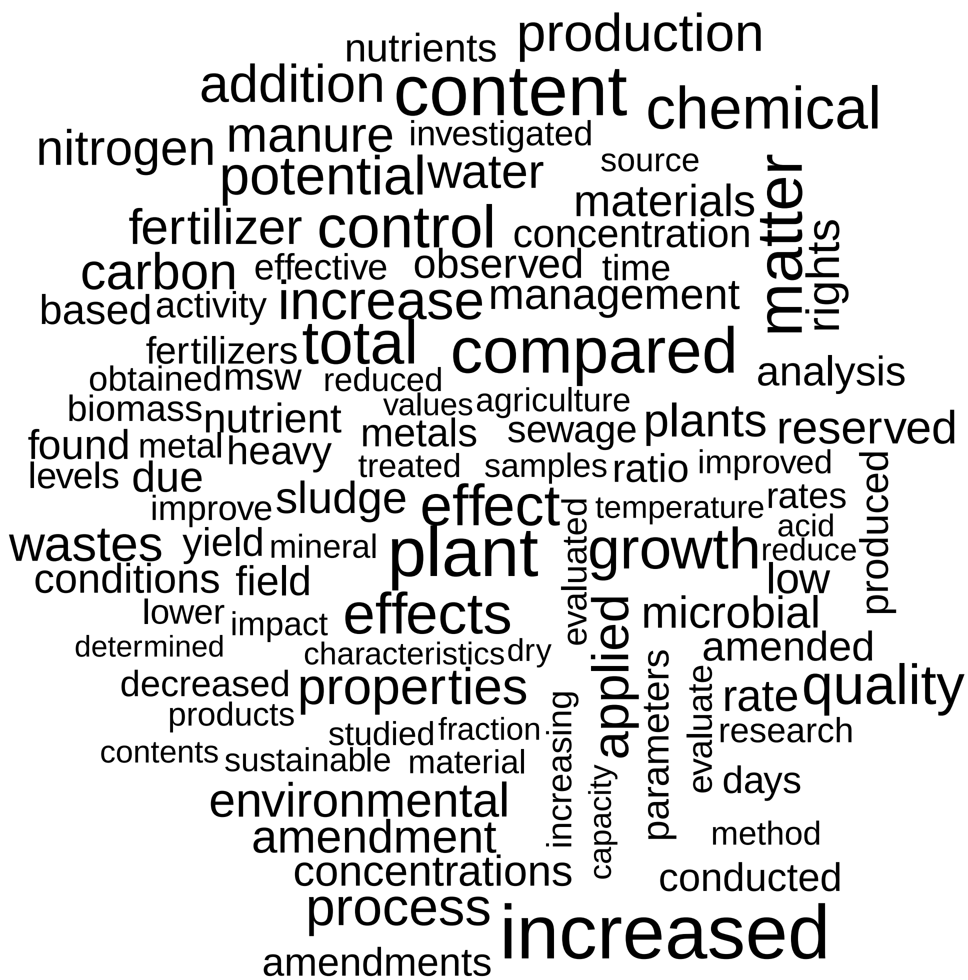


Figure 9: Word cloud of the top 100 terms from the abstracts of the 115 reviewed articles on MSWC. Common stop-words were removed and terms lower-cased; word size is proportional to relative frequency. Source: Web of Science (WoS) Core Collection, 1985–2024. Abbreviations: MSWC = municipal solid-waste compost.

Following Text Mining, a Latent Dirichlet Allocation (LDA) model was applied to further structure the analysis of the research field (Fig. S4). Topic modeling was used here as an organizational tool to group documents based on shared vocabularies and to support transparent screening of the literature, rather than to infer semantic meaning from individual terms. Topics are therefore described through their highest-probability terms ($\beta$) and interpreted cautiously. Some topics show associations with specific research domains. For example, the recurrent presence of the term "microbial" in topics no. 2 and no. 6 suggests that these clusters include studies addressing soil biological activity. Similarly, the occurrence of "biochar" in topics no. 5 and no. 13 indicates the inclusion of studies investigating biochar use in combination with MSWC. Topics no. 12 and no. 14 include contamination-related terms such as "metal" and "heavy", respectively, consistent with studies focusing on heavy metal dynamics in compost-amended soils. These associations are reported as indicative patterns based on topic composition rather than as definitive thematic classifications.

After topic-based screening and full-text assessment, a total of 115 articles were retained for detailed analysis. These articles address crop productivity (85/115, ~74%) and soil fertility (70/115, ~61%), with some studies covering both objectives; consequently, the total number of objective occurrences (155) exceeds the number of articles. Regarding trial duration, 60 studies (52%) covered one year, 23 focused on two years (20%), 19 on three years (16%), and 13 extended beyond three years (12%). Production systems were predominantly focused on arable crops (57/115, ~49%), followed by horticulture (45/115, ~39%) and orchards (15/115, ~13%). Two articles (Jorge-Mardomingo et al. 2015, 2013) focus on the effects of MSWC on soil properties without targeting a specific crop. The total number of production systems analysed (119) is higher than the total number of articles (115) because two studies evaluated more than one system: one (Sánchez-Monedero et al. 2019), with a trial duration of three years, assessed the effects of MSWC on a crop rotation that included arable crops, horticultural crops, and orchard systems; the other (Gallardo-Lara et al. 2006), with a duration longer than three years, simultaneously investigated both arable and horticultural systems. Most of the studies (86, ~75%) were field-based experiments, while only 29 are pot experiment (25%). A total of 97 experiments were conducted under open field conditions and 19 in greenhouses. The sum exceeds the number contained in database E because Sánchez-Monedero et al. (2019) evaluated multiple agroecosystems.

### 4.5.4 Crop Productivity: within one year experiments

The limited number of publications (85, Table 1) prevents drawing broadly applicable and globally valid conclusions on the impacts of MSWC application on crop productivity. Nevertheless, some insights can be derived from consolidated findings, though constrained to specific pedo-climatic and production conditions.

As shown in Table 1, 60 out of the papers contained in database E (Fig. 6) report findings from

Table 1: Summary of the 115 reviewed articles contained in database E and grouped by main objective, trial duration, production systems, experimental methods, and study conditions. The last row reports the total number for each group but for main objective, production systems, and study conditions the total exceeds 115 because some studies encompass multiple groups.

| | Main objective | | Trial duration | | Production systems | | Experimental methods | | Study conditions | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Crop productivity | 85 | 1 year | 60 | Arable crops | 57 | Field experiment | 86 | Open field | 97 |
| | | | 2 years | 23 | Horticulture | 45 | | | | |
| | Soil fertility | 70 | 3 years | 19 | Orchards | 15 | Pot experiment | 29 | Greenhouse experiment | 19 |
| | | | Over 3 years | 13 | No crop | 2 | | | | |
| Total | | 155 | | 115 | | 119 | | 115 | | 116 |

one-year experiments, where 48 specifically focus on the effects of MSWC on crop productivity.

The results describe the beneficial effects of MSWC on arable crops, highlighting its potential to enhance yield and plant performance across different species and environmental conditions. For instance, Prabpai et al. (2009) found 85% increase in *Zea mays* (corn) yield when applying rates of MSWC/soil vol/vol from 20 to 80%, with the highest yield observed at 60%. In a field study, Ulm et al. (2019) reported grain yields of 1.9 t ha$^{-1}$ in unamended plots and 7.8 t ha$^{-1}$ in MSWC treated plots (+311%). In a pot trial, Okoli et al. (2022) found that 100% MSWC increased plant height from 127 cm in untreated plots to 162.6 cm (+28%), grains per cob from 220 to 350 (+59%), and cob weight from 100 g to 250 g (+150%); 75% compost yielded 152.4 cm (+20%), 320 grains (+45%), and 200 g (+100%) relative to the control. Similar positive effects have been reported for cereals such as *Oryza sativa* (rice), **Triticum aestivum* (common wheat) and *Triticum durum* (durum wheat) particularly in challenging soil conditions. Zaman et al. (2020) observed that MSWC distribution significantly increased the productivity of rice and wheat in Pakistani saline-sodic soils: the highest yields were recorded for rice straw (1102 g m$^{-2}$), rice grain (605 g m$^{-2}$), wheat straw (984 g m$^{-2}$), and wheat grain (505 g m$^{-2}$). The increase in yield (+67% in rice straw, +132% for rice grain, +26% for wheat straw and +48% for wheat grain, respectively), was most pronounced when the compost underwent anaerobic decomposition, highlighting the role of composting techniques in influencing crop performance. Supporting these findings, Lakhdar et al. (2009) found that applying MSWC at rates as high as 80 t ha$^{-1}$ resulted in an 87% increase in grain yield of durum wheat compared to unamended plots. In the second year of another of their experiments (Lakhdar et al. 2010), biomass production also increased significantly, to 48% and 78% observed at compost application rates of 40 and 100 t ha$^{-1}$, respectively. More recently, Gadaleta et al. (2022) confirmed that MSWC increased durum wheat yield by 25.52% and 30.92% in two different cultivars. Martinez et al. (2022) observed an increase in buckwheat biomass production, seed yield, seed quality, and nutrient content when using MSWC instead of mineral fertilization in a greenhouse study conducted on both clayey and sandy soils. The study reported average yields of 34.4 g/plant for biomass and 10.6 g/plant for seeds. However, the effectiveness of MSWC appears to be crop-specific and dose-dependent. Notably, the only exception among the arable crop studies reviewed is Kchaou et al. (2018), who found that MSWC application did not lead to significant increases in *X Triticosecale Wittmack* (triticale) plant height, leaf number, or chlorophyll index, suggesting that the effectiveness of compost may vary with environmental conditions, crop species and application rate.

The advantages of MSWC application extend beyond cereals, as observed in oilseeds and forage crops. Montemurro et al. (2005), observed that MSWC produced *Helianthus annuus* (sunflower) yields comparable to those obtained with mineral fertilizers, particularly for oil and protein content, while Rashtbari et al. (2020) reported that MSWC improved plant height, stem diameter, and grain yield in *Brassica napus* (canola) under both well-watered and drought stress conditions. In forage crops, MSWC has shown potential in mitigating the adverse effects of salinity and enhancing biomass production, although responses tend to be crop- and soil-specific. Mbarki et al. (2020) reported improved plant growth when irrigation with saline water (50-100 mM NaCl solution) was combined with MSWC application. Similarly, Ouni et al. (2014) observed that MSWC improved both biomass production and photosynthetic efficiency in forage species grown under saline soil conditions. However, the effectiveness of MSWC varied with soil type, as demonstrated by Mbarki et al. (2008): in clay soils, compost application increased *Medicago sativa* (alfalfa) yield by 15% at 10 t $ha^{-1}$, 24% at 20 t ha-1, and 18% at 40 t $ha^{-1}$. Thus, a compost dose higher than 40 t $ha^{-1}$ was not necessary to improve yield in such soil. In contrast, a decline in productivity was observed in sandy soils with increasing compost rates, likely due to enhanced nutrient leaching and salinity accumulation. Positive outcomes were also reported in *Lolium perenne* (ryegrass), with Soumaré et al. (2003) documenting yield increases of 10% and 17.5% at application rates of 50 t $ha^{-1}$ in two distinct tropical soils. The positive impact of MSWC is also evident in leguminous crops, as Ozdemir (2005) observed that *Cicer arietinum* (chickpea) responded best to MSWC application at 50 t $ha^{-1}$, significantly increasing seed yield. Confirming the crop- and condition-dependent nature of MSWC responses, as already noted in triticale (Kchaou et al. 2018), Sifola et al. (2025) reported that compost application under deficit irrigation reduced *Nicotiana tabacum* (tobacco) yield, despite improvements in soil water retention and N use efficiency (NUE), an outcome consistent with temporary N immobilization and lower plant N recovery during rapid growth, coupled with the crop's high sensitivity to water stress during key phenophases.

The application of MSWC has shown promising results in various horticultural production systems, enhancing biomass production, vegetable yield, and overall crop performance. Among leafy vegetables, Papafilippaki et al. (2015) reported that applying 60 t $ha^{-1}$ of MSWC enhanced *Cichorium spinosum* (spiny chicory) yields in sandy soils, while De Nobile et al. (2021) showed that, despite the same nitrogen doses being applied, MSWC led to higher biomass yields in *Brassica oleracea* var. *italica* (broccoli) and *Lactuca sativa* (lettuce) than urea, especially at the highest application rates (6.2 ton ha-1). Castaldi et al. (2023) also demonstrated that MSWC application (35 t $ha^{-1}$) enhanced *Beta vulgaris* var. *cicla* (Swiss chard) yields to levels comparable with mineral fertilization. By contrast, Coria-Cayupán et al. (2009) reported a slight yield reduction (-7%) in lettuce cultivated with MSWC. In the case of solanaceous crops, Raji et al. (2016) observed that increasing the proportion of MSWC in media blends improved the growth and development of *Capsicum annuum* (pepper) seedlings, with the highest compost levels (50 % vol/vol) yielding the best results across several growth metrics. Consistently, Leogrande et al. (2016a) found that MSWC increased *Solanum lycopersicum* (tomato) yield by 9% compared to mineral fertilizers in Southern Italy. Tzortzakis et al. (2020) confirmed that MSWC additions (from 5% up to 40% vol/vol) in the growing medium improved tomato plant biomass and yield although excessively high ratios reduced fresh weight. Bhardwaj et al. (2023) also observed increased yields in tomato and *Solanum melongena* (brinjal) following MSWC amendments, with the best results obtained at a compost application rates of 25% MSWC/soil vol/vol. Leguminous horticultural crops also benefit from MSWC application, as demonstrated by Soobhany et al. (2017) which found that *Phaseolus vulgaris* (green bean) plants grown in soil mixtures containing 40% vol MSWC produced significantly higher legume yields compared to the control. Likewise, Martinez et al. (2021) found that MSWC (115 g/L) increased *Vicia faba* (faba bean) biomass production by 38% in sandy soil compared to the control. Wilton et al. (2020) demonstrated that green bean yields increased linearly with compost application rates (up to 30% vol/vol), where all growth parameters, including leaf area and pod number, responded positively without reaching the yield plateau. In horticultural fruit crops, Sayğı (2023) found that *Fragaria x ananassa* (strawberry) grown with 4 t $ha^{-1}$ of MSWC had the highest fruit weight and yield. Likewise, Villena et al. (2018) observed a significant improvement in *Cucumis melo* (melon) yield under field conditions when MSWC was applied, especially at moderate rates (13 t $ha^{-1}$), which proved sufficient to maximize yield without exceeding environmental risk thresholds.

Regarding orchards, Basso-Figuera et al. (1995) found that MSWC increased *Carica papaya* (papaya) yields proportionally to the application rate, with no significant changes in leaf nutrient levels. Similarly, Blanco et al. (2024) evaluated the use of MSWC as mulch in a vineyard (*Vitis vinifera*) located in Southern Italy and observed that its application helped stabilize soil temperature, reducing heat stress on roots and improving water retention. These effects, although not quantified in terms of yield, suggest enhanced vine health and potential support for grape production. In another orchard crop study, Schulz et al. (2012) tested urban waste compost as a fertilizer source for *Jatropha curcas* (physic nut) under greenhouse conditions. They found that MSWC application increased plant growth parameters such as height, leaf area, and chlorophyll index up to a dose of 200 L $m^{-3}$, beyond which no further benefits were observed. In a semiarid shrubland setting, Cuevas et al. (2000) reported that MSWC increased total plant cover and biomass production of native species, with the most notable biomass gains occurring at 80 t $ha^{-1}$, confirming the positive role of MSWC in promoting vegetative productivity even under degraded conditions.

### 4.5.5 Crop Productivity: two year experiments

In this review, of the 115 articles analysed, 25 cover a period of 2 years, as shown in Table 1. Notably, 17 of these investigate the impact of MSWC on crop productivity.

These experiments consistently observed the benefits of MSWC across different cropping systems, improving plant growth, yield, and nutrient availability. Regarding arable crops, in the context of corn production, Hossain et al. (2024) found that the application of MSWC (5, 10, and 15 t $ha^{-1}$) had a positive impact on plant growth and yield in their two-year field study in Saudi Arabia. Effects were further amplified when MSWC was combined with NPK, with the strongest responses at the highest application rate. Similarly, Wolkowski (2003) reported that mature MSWC significantly increased corn grain yields compared to a less mature one. The superior performance of mature MSWC was attributed to its lower N immobilization and the gradual release of N, which provided a continuous nutrient supply throughout the growing season. In tobacco cultivation, Sifola et al. (2024) showed that MSWC (12.5 t $ha^{-1}$) improved plant growth parameters such as leaf emergence rate and crop growth rate, ultimately enhancing the yield of cured product and resource use efficiency. For cereals, Montemurro (2009) found that MSWC increased winter wheat yields by 12% compared to the control. However, the yields achieved with MSWC were lower than those obtained with mineral fertilizer and MSWC-mineral fertilizers blends. These observations align with findings from Mehdi et al. (2015), who reported that an 80/20 MSWC/mineral fertiliser mixture is capable to meet the crop's nutrient needs on a par with minerla fertiliser alone, but can significantly increase biomass production and grain yield in a rice/common wheat rotation. Jorge-Mardomingo et al. (2015) also noted that high doses of MSWC (160 t $ha^{-1}$)enhanced yield stability in cereals under Mediterranean conditions by ensuring a more gradual nutrient release and reducing nitrate leaching compared to less stable organic amendments.

In horticultural systems, for example in lettuce production, Leogrande et al. (2013) and Testani et al. (2020) observed that MSWC, either alone or in combination with agroecological practices like cover cropping, contributed to stable yields and improved NUE. Leogrande et al. (2013) noted that MSWC led to lower marketable yields than stable manure but both treatments enhanced soil fertility and contributed to yield sustainability, Testani et al. (2020) reported that MSWC, when integrated with roller crimper-based no-till systems and legume cover crops, supported consistent lettuce yields across two cropping cycles. For solanaceous crops, Clark et al. (2000) investigated MSWC effects on drip-irrigated and sub-irrigated autumn-grown green peppers and spring-grown fresh market tomatoes between 1992 and 1994. Initially, they found that the incorporation of immature MSWC (67 and 134 t ha$^{-1}$) reduced growth and yields of pepper in the autumn 1992 However, by the spring of 1993, the fully decomposed MSWC significantly enhanced plant size and fruit yields in tomatoes. Compared to control plots the application of 134 t ha$^{-1}$ of MSWC, increased tomato yields by 27% in the spring of 1993 and by 18% in 1994. Similarly, pepper yields improved by 17% in the autumn of 1993. These results are in line with the findings of Ozores-Hampton et al. (1994), who reported improvements in growth, nutrient concentrations, and yields in tomato and *Cucurbita maxima* (squash) crops after MSWC application.

However, other studies have shown less favourable outcomes under certain conditions: Farneselli et al. (2022) found that MSWC alone did not meet the N requirements of processing tomato and *Brassica oleracea* var. *botrytis* (cauliflower), leading to reduced aboveground biomass accumulation compared to mineral fertilization. Nevertheless, the early application of MSWC partly mitigated these negative effects.

The benefits of MSWC extend to perennial orchard systems as well. Aguilar et al. (1997) found significant increases in the yields of *Olea europaea* (olive), vine, and *Citrus* spp. (citrus) crops following the application of MSWC. Olive orchards showed a 50% increase in fruit yield, while vineyards experienced a 30% rise with applications of 30 t ha$^{-1}$, while citrus crops exhibited a 17% improvement in yield. These findings are consistent with those of Baldi et al. (2014), who observed that in vineyards, the use of MSWC significantly improved yield and nutrient uptake in *Vitis vinifera* L. cv. Sangiovese, particularly when used in combination with humic substances.

### 4.5.6 Crop Productivity: three year experiments

The following section presents evidence from the subset of 15 articles out of 19 with three-year experiments (Table 1) that specifically investigated crop productivity.

In corn production, Golabi et al. (2021) found that MSWC improved yields over three growing seasons in northern Guam. During the rainy season, plots treated with 250 t ha$^{-1}$ of compost yielded more than those treated with mineral fertilizers. Supporting this, Moretti et al. (2020) also reported that the substitution of mineral fertilizers with MSWC in continuous maize cropping systems led to a 55% yield increase by the third year, emphasizing the compost's effectiveness in arable summer crops. Conversely, in *Solanum tuberosum* (potato) cultivation, Warman et al. (2011) found that MSWC treatments did not consistently influence marketable tuber yield. These findings suggest that the effectiveness of MSWC may vary depending on the crop and environmental conditions. In cereals system, Mousavi et al. (2017) reported that MSWC significantly enhanced rice yields, while Meena et al. (2022) found that MSWC improved soil structure in saline soils, leading to significantly higher grain yields in a *Brassica juncea* (mustard)/*Pennisetum glaucum* (pearl millet) cropping system in India. The beneficial effects of MSWC were also evident in some combined cereal-forage systems. Mbarki et al. (2022) observed that MSWC significantly boosted biomass yield in *Sorghum bicolor* (sorghum) during the second harvest and in *Medicago sativa* (alfalfa) during the third one under saline conditions over three harvest periods. Similarly, Montemurro et al. (2006) reported that MSWC increased the mean cumulative dry weight of *Dactylis glomerata* (cocksfoot) by 20.85% compared to the control in a field experiment in Southern Italy. In alfalfa, the cumulative dry weight remained consistent across all treatments. Further supporting these findings, Salis et al. (2024) observed significant increases in dry matter production and crude protein content in rainfed forage barley (*Hordeum vulgare*) with the application of MSWC. Zając and Skrajna (2024) added to these findings, showing that in perennial grasses like *Miscanthus sinensis* (Chinese silver grass), yields increased steadily, with the highest yields recorded in MSWC treated plots (17.5 t $ha^{-1}$).

In horticultural systems, positive effects were also evident. Alashty et al. (2011) found that the continuous application of MSWC (20 and 40 t $ha^{-1}$ ) significantly enhanced plant growth by modifying soil chemical properties. They observed that higher compost rates increased root and shoot biomass in *Raphanus sativus* (radish) and lettuce, with the most notable effects occurring on the third year. Similarly, Warman et al. (2009) on winter squash cultivation in Nova Scotia, Canada, found that higher application rates (up to 72 t $ha^{-1}$) significantly improved yields, particularly in the third year, reaching levels similar to those obtained with mineral fertilizers. Complementing these results, Sánchez-Monedero et al. (2019) reported that in greenhouse-grown tomatoes, at the end of the third year MSWC application increased fruit weight, diameter, and hardness by 16%, 9%, and 8%, respectively.

In orchard systems, Bona et al. (2022) found that MSWC applied in intensive *Malus domestica* (apple) orchards showed a linear increase in productivity over time, with more evident effects than matured manure. Similarly, Sánchez-Monedero et al. (2019) found that MSWC (10.9 t $ha^{-1}$) in vineyards enhanced grape must acidity, a quality parameter important in wine production.

### 4.5.7 Crop Productivity: more than three years

Of the 115 articles analyzed in this review, 13 focus on the long-term effects of MSWC, with study durations exceeding three years. The following section will focus on six studies that specifically investigated crop productivity.

Leogrande et al. (2016b) found that MSWC (50 t $ha^{-1}$) increased maize grain yield by 11% compared to mineral fertilizers in Southern Italy. Under saline irrigation, they observed a 19% higher yield with MSWC compared to mineral fertilizer and control treatments. Similarly, Hemmat et al. (2010), in arable cropping systems, found that long-term MSWC applications (from 25 to 100 t $ha^{-1}$) significantly enhanced wheat and corn yields, mainly due to increased SOM and N availability. In a eleven year field trial in Denmark, Gómez-Muñoz et al. (2017) reported that a mean of 20.7 t $ha^{-1}$ of MSWC applied annually contributed to increase soil N stocks and SOM content in an oat (*Avena sativa*) cropping system. However, crop responses were limited by low NUE, suggesting that compost quality and synchronization with crop N demand play a critical role in determining yield outcomes.

For horticultural crops, in a four year trial, Morra et al. (2010) found that up to 45 t $ha^{-1}$ had no significant effect on tomato or green bean yields in a greenhouse experiment. However, lettuce yields increased with applications at 30 and 45 t $ha^{-1}$, once more highlighting the crop-specific effects of MSWC.

In orchard systems, MSWC at 10 t $ha^{-1}$, successfully replaced mineral fertilizers in commercial Prunus persica (peach) orchards, maintaining or even increasing fruit yield over a nine-year period (Baldi et al. 2010). Likewise, compost mulch improved grapevine productivity, mainly through enhanced soil moisture retention and nutrient availability, though concerns regarding heavy metal accumulation were noted in some cases (Pinamonti 1998).

### 4.5.8 Soil fertility: within one year experiments

Developing broad and globally valid conclusions on the effects of MSWC application on soil fertility requires a careful examination of available studies, which are often limited to specific pedo-climatic and management conditions. As reported in Table 1, the articles reviewed that investigated MSCW effects on soil fertility are 70. While in the previous sections on crop productivity the effects were categorized across arable crops, horticulture, and orchards, here the effects will be grouped according their physical, chemical, and biological aspects of soil fertility.

Of the 115 articles analysed in this review, 60 focus on the short-term effects, i.e. on the results obtained after a single year. The following section will highlight some of the 35 studies that specifically investigated soil fertility.

The ability to enhance soil's physical properties is a key benefit of MSWC applications, and that seems particularly true for water retention and soil structure. For instance, Zgallai et al. (2024) compared the water retention properties of MSWC distribution with a commercial water-retaining product;at the beginning of the dry season, soil moisture tension was found lowest in compost-amended plots (48 cb), higher in commercial product treated ones (61 cb) and hjghest in the control (83 cb). Since tension is expressed as positive centibar values, lower values indicate wetter soil and more readily available water. At the end of the field trial, they also observed that compost application increased soil moisture, total N, and SOM by 9%, 50%, and 27% respectively, compared to the commercial water-retaining product. Similarly, Taheri Soudejani et al. (2020) found that cumulative drainage content decreased from 35.68% in the control to 10.95% in the MSWC treatment (40 t $ha^{-1}$). Additionally, they observed an increase in topsoil water content by up to 9.08% in the compost-treated group compared to the control. In horticultural systems, Mylavarapu and Zinati (2009) observed improved moisture retention under drought conditions in *Petroselinum crispum* (parsley) cultivation, while Cercioglu (2019) noted enhancements in overall soil structure. These findings are in agreement with these obtained by Sifola et al. (2025), which reported an increased water-holding capacity after MSWC application (18.5 t $ha^{-1}$), contributing to greater resilience under water stress. Schulz et al. (2012) also observed improved soil physical conditions during early growth stages of physic nut (*Jatropha curcas* L.) in a pot trial. In vineyard systems, Blanco et al. (2024) found that MSWC mulching enhanced microbial decomposition activity (Tea Bag Index) and reduced the diurnal soil-temperature amplitude at 20 cm; these conditions may favor longer-term improvements in soil structure.

In addition to physical benefits, MSWC significantly contributes to soil chemical fertility by increasing SOM, nutrient availability, and by modifying some critical chemical properties. Zaman et al. (2020), in a rice-wheat rotation experiment conducted on saline-sodic soils, reported substantial increases in SOM after MSWC application (34.3 t $ha^{-1}$): +64.10% after rice and +86.32% after wheat. Compost also reduced soil electrical conductivity (EC) (-35.61%) and SAR (-45.22%) after rice harvest, with even greater reductions following wheat. Montemurro et al. (2005), working on a silty clay soil in Southern Italy, observed elevated levels of available P and SOM in sunflower-cultivated soils, while Lakhdar et al. (2011) observed that, in a sandy soil from semi-arid Tunisia, MSWC enhanced nutrient levels in saline soils, particularly N and K, helping to counteract the negative effects of salinity. Kasthuri et al. (2011) further confirmed these benefits in their pot experiment on *Vigna radiata* (green gram) and *Trigonella foenum-graecum* (fenugreek), finding increases in essential nutrients levels. Martinez et al. (2022) reported increasesin SOM (+32%), total N (+62%), and pH (from 7.45 to 8.05) in MSWC treated plots compared with unamended controls, both under greenhouse and open-field conditions, Martinez et al. (2021) observed improved NUE in faba beans, lowering the need for mineral fertilizers. Regarding to horticulture production systems, Mylavarapu and Zinati (2009) reported increases in total organic carbon (TOC) and N, while Cercioglu (2019) found gains in total N, available P and K. Erana et al. (2019) also noted that MSWC increased soil pH and TOC, supporting enhanced root growth and nutrient absorption in *Allium cepa* (onion) crops. Leogrande et al. (2016a) showed that MSWC reduced the Na concentration while increasing those of Ca and K in tomato cultivation on saline soils. Guerini et al. (2006) and Leogrande et al. (2016b) further confirmed increases in SOM (+20–30%), cation exchange capacity (CEC), and nutrient availability, while Soumaré et al. (2003) emphasized that co-application with mineral fertilizers, enhanced N and P uptake. In subarctic soils, Wilton et al. (2020) reported significant increases in P and K availability for bush beans, though excess accumulation of these nutrients at high compost rates (30% MSWC/soil vol/vol) raised concerns about nutrient saturation. Similar effects were noted by Cuevas et al. (2000), who observed higher N, available P, and exchangeable K levels in a degraded semiarid shrubland.

Nevertheless, several studies cautioned against excessive MSWC application, particularly in sandy or degraded soils. Mbarki et al. (2008) and Bhardwaj et al. (2023) reported increased salinity and accumulation of Cd, Cu, Zn, and Pb. These effects were particularly evident in the sandy soils of Tunisia (pH 8.0) studied by Mbarki et al. (2008), and in the sandy alluvial soils of India (initial pH 8.48) examined by Bhardwaj et al. (2023), where compost rates exceeding 25% MSWC/soil vol/vol markedly increased SOM and available P while altering N and K dynamics.. Fagnano et al. (2011) also highlighted the increased bioavailability of Pb and Zn, underlining the need for careful monitoring.

Beyond chemical and physical improvements, MSWC substantially contributes to soil biological quality and ecosystem functioning. Hodson et al. (2021) demonstrated that MSWC altered microbial community composition and increased bacterial evenness in almond orchards, with a higher relative abundance of taxa involved in carbon degradation and distinct associations between bacterial groups and bacterial-feeding nematodes. These shifts in microbial and nematode communities enhanced nutrient cycling and contributed to long-term soil fertility. Lakhdar et al. (2010) reported marked increases in enzymatic activity following compost application: dehydrogenase (+107%), $\beta$-glucosidase (+43%), and phosphatase (+20%), which were interpreted as positive outcomes reflecting enhanced microbial metabolism and nutrient turnover, thereby improving the biological quality of salt-affected soils. Elbl et al. (2019) observed increased microbial respiration following MSWC application, indicating enhanced substrate mineralization and $CO_2$ release; although this reflects short-term stimulation of microbial activity, it also implies that a relevant share of the added C may be lost rather than stabilized. Nonetheless, they reported that part of the carbon input contributed to soil C sequestration, suggesting a potential, though limited, long-term benefit for biological fertility. De Varennes et al. (2010) documented higher activities of soil enzymes such as $\beta$-glucosidase and phosphatase in degraded mine soils, with improved activity referring to a recovery of biological functioning compared to untreated controls, thereby reinforcing the restorative potential of MSWC. Consistent with these findings, in a field experiment under semi-arid climate, Jorge-Mardomingo et al. (2013) reported that a single high dose (160 t $ha^{-1}$) was able to significantly increae microbial biomass C,up to 1369.1 mg C $kg^{-1}$, to maintain high basal respiration rates, and to enhance enzymatic activities such as $\beta$-glucosidase, urease, and phosphatase. Unlike sewage sludge treatments, which induced initial microbial stress and seasonal fluctuations, MSWC was capable to keep a stable biological activity and a low metabolic quotient (5.04 ng $O_2\,\mu g^{-1}$ Microbial Biomass Carbon $h^{-1}$)than sewage sludge, indicating efficient microbial function and low ecological disturbance. Terrile et al. (2024) confirmed the improved physical, chemical, and biological soil properties due to MSWC (40 t $ha^{-1}$), while detecting low residual pollutants: Pb and As in some leaf-based composts, veterinary pharmaceuticals and trace pesticide residues in irrigation water and soils. Notably, contaminant concentrations in vegetable tissues were generally below admissible limits or not detectable. Similarly, Oguntade et al. (2019) found improvements in soil fertility indicators (e.g., total N, TOC, available P), while maintaining heavy metal concentrations within safe thresholds.

However, potential trade-offs must be acknowledged. Novinscak et al. (2022) reported increased $N_2O$ emissions in compost-treated soils, pointing to the need for integrated management approaches that optimize nutrient use while minimizing environmental impacts.

### 4.5.9 Soil fertility: two year experiments

Among the 115 articles reviewed, 25 include experiments lasting two years, as shown in Table 1. The following sections summarize the findings from eleven papers that focus the medium-term effects on soil fertility.

In terms of physical improvements, Aguilar et al. (1997) reported that MSWC application in olive, vine, and citrus orchards led to significant enhancements in soil structure. Compost reduced bulk density, increased water-holding capacity, improved aggregate stability, and promoted better water infiltration, thereby increasing the soil's resistance to erosion. Similarly, Leogrande et al. (2013), who reported increased soil quality parameters. These findings are echoed by Moraes et al. (2014) observed that amounts up to 75 $m^3$ $ha^{-1}$ distributed over two years under no-tillage in corn and sunflower cultivation, were able to improve soil aggregation compared to mineral fertilization in a Rhodic Hapludox, and by Testani et al. (2020), who highlighted the role of MSWC, especially when combined with cover crops, in sustaining soil structure under organic lettuce systems.

From a chemical perspective, Montemurro et al. (2007) found that MSWC application (68 t $ha^{-1}$) resulted in a substantial (+37%) TOC increase after two years under *Beta vulgaris* (sugar beet) cultivation. Leogrande et al. (2013) confirmed these findings in lettuce cultivation, observing that MSWC improved the extractable fraction of TOC more than other organic fertilizers. Similarly, Moraes et al. (2014) noted that both TOC and total N increased linearly with MSWC application rates (from 25 to 100 $m^3$ $ha^{-1}$). Testani et al. (2020) also found that the use of MSWC, especially when combined with *Vicia* spp. (vetch) cover crops, supported the preservation of SOM and N levels across two consecutive growing seasons. Moreover, Aguilar et al. (1997) found that compost enhanced the availability of macro and micronutrients and increased soil CEC, indicating improved nutrient retention and supply potential. Rashtbari et al. (2020) also reported increased levels of available P, K, and Zn following the combined application of MSWC and mineral fertilizers (20:80) in a rice-common wheat system, along with a slight reduction in pH and SAR. Gallardo-Lara et al. (2006) further contributed to this evidence by showing that MSWC (20 and 80 t $ha^{-1}$) improved the phytoavailability of Fe and Mn in calcareous soils, enhancing micronutrient accessibility.

In relation to N dynamics and nutrient release patterns, Wolkowski (2003) highlighted that 6~17% of the N in mature MSWC became bioavailable within the first year of application. This gradual and consistent release of nutrients reduced the risk of $NO_3^-$ leaching, thus mitigating adverse environmental impacts. These findings are supported by the positive linear relationship between application rates and total N availability reported by Moraes et al. (2014), reinforcing the role of MSWC in enhancing long-term nutrient supply. Jorge-Mardomingo et al. (2015) similarly noted that MSWC, when compared to other organic amendments, provided a more stable N release pattern under Mediterranean pedoclimatic conditions, reducing the risk of groundwater contamination. Rupasinghe and Leelamanie (2020) further emphasized the importance of site-specific MSWC application, noting that while it improved nutrient status, it also increased soil pH and EC.

### 4.5.10 Soil fertility: three year experiments

The following section presents evidence from the subset of 12 articles out of 19 with three-year experiments (Table 1) that specifically investigated soil fertility.

From a physical point of view, Meena et al. (2022) observed that MSWC applications in saline soils enhanced TOC, aggregate stability, water retention capacity, and pore size distribution, leading to a marked reduction (-75%) in soil salinity. Bona et al. (2022), in a study on intensive apple orchards, observed that MSWC improved the physical structure of soils by increasing porosity and reducing compaction in the surface layers, especially under reduced tillage. Similarly, Zając and Skrajna (2024) demonstrated that MSWC application in marginal soils cultivated with Chinese silver grass significantly improved soil structure and moisture retention, supporting plant development in nutrient-poor environments; these effects are likely context-dependent and may reflect a synergistic interaction between compost inputs and crop traits (e.g., rooting architecture, residue inputs), rather than MSWC alone.

From a chemical perspective, numerous studies highlight MSWC ability to enrich SOM and nutrient content. Golabi et al. (2021) observed that MSWC-treated plots in Guam had significantly higher SOM than those treated with mineral fertilizers. Likewise, Montemurro et al. (2006) found that three consecutive years of MSWC application significantly increased TOC in both alfalfa and cocksfoot plots. The study also observed that MSWC increased the C and N soil content, contributing to enhanced fertility. Similarly, Salis et al. (2024) reported that MSWC enhanced several critical soil properties, including TOC, total N, available P, exchangeable K, and Mg. However, they also noted that MSWC increased the percentage of exchangeable Na and EC, highlighting the importance of potential trade-offs when using compost as a soil amendment. In the maize system, Moretti et al. (2020) reported that although the total N and Mineral-Associated Organic Matter (MAOM) did not vary significantly over the three-year period, MSWC application led to a consistent increase in more labile organic fractions (free Particulate Organic Matter, fPOM, and occluded particulate Organic matter, oPOM) in the topsoil, indicating a gradual improvement in nutrient dynamics and SOM cycling. Further evidence comes from Leogrande et al. (2014), who observed increases in TOC and in humic and fulvic acid fractions after three years of MSWC use, indicating enhanced SOM quality and stability. Similarly, Zaccardelli et al. (2021) documented cumulative changes in most soil chemical parameters in horticultural systems following three consecutive years of MSWC application, with the exception of P and B, which remained stable. Alashty et al.

(2011) found that applying MSWC (40 t $ha^{-1}$) lowered soil pH while increasing TOC and EC. Sánchez-Monedero et al. (2019), analyzing multiple systems including vineyards, arable crops, and greenhouses, reported that MSWC application increased TOC and nutrient availability (especially K, N, and extractable OC), while slightly rising pH in acidic soils and stimulating soil microbial activity. In line with these findings, Bona et al. (2022) noted significant increases in soil total N and SOM with MSWC use (20 t $ha^{-1}$) in apple orchards, with effects becoming more pronounced over time.

The improvement of soil's biological qualities and functionality was evident across several cropping systems. Sánchez-Monedero et al. (2019) observed that MSWC-treated soils showed enhanced microbial respiration, an increase of denitrification, and improved nutrient cycling, suggesting a more active microbial community with higher capacity to transform and recycle nutrients, thereby supporting soil fertility functions. These results were especially pronounced in tomatoes under greenhouse and in vineyards, where MSWC also contributed to maintain microbial biodiversity. Similarly, in the maize cultivation studied by Moretti et al. (2020), MSWC application helped to mitigate $N_2O$ emissions compared to conventional mineral fertilization, pointing to a better ecological performance of the soil.

### 4.5.11 Soil Fertility: more than three years experiments

The following section presents evidence from the subset of 12 articles out of 13 with three-year experiments (Table 1) that specifically investigated soil fertility.

Improvements in soil structure and water retention have been observed in response to increased SOM. For instance, Guerini et al. (2006) reported a 30% increase in SOM after five years of continuous MSWC applications (30 t $ha^{-1}$ each year), which was accompanied by better soil structure and enhanced water-holding capacity. In vineyard systems, Pinamonti (1998) similarly observed that MSWC mulch increased soil porosity and water retention capacity, contributing to improved physical conditions in the root zone. In arable cropping systems, long-term applications of MSWC (7.1 t $ha^{-1}$) significantly enhanced soil physical properties as well. Hemmat et al. (2010) found that compost-amended soils doubled their TOC, which was associated with reduced bulk density and improved consistency limits-key indicators like shrinkage limit (%), plastic limit (%) and friability index (%). Peltre et al. (2015) confirmed changes in physical structure led to practical agronomical benefits, such as reduced draught force during tillage and lower tractor fuel consumption, thus improving soil manageability.

As for chemical fertility, several studies emphasize the MSWC role in increasing TOC and N levels. In arable systems, Leogrande et al. (2016b) found a significant increase (+25 %) in the soil's TOC after MSWC application (50 t $ha^{-1}$), while Montemurro et al. (2010) found that MSWC amendments contemporarily boosted soil total N, TOC, extractable OC, and humified carbon fractions, compared to the initial conditions and to the application of mineral fertilizer. Gómez-Muñoz et al. (2017) supported these findings, showing that MSWC repeated applications for eleven years (20.7 t $ha^{-1}$) significantly improved soil total N and total C compared to mineral fertilization. Compost also enhanced nutrient availability, as evidenced by higher concentrations of available N, P, and K, along with increased soil pH and CEC (Guerini et al. 2006). In horticultural systems, Morra et al. (2010) noted similar chemical improvements with increasing compost application rates (from 15 to 45 t $ha^{-1}$), though they also found that the efficiency of TOC conversion into stable soil pools peaked at 30 t $ha^{-1}$ (36% efficiency) and declined at higher MSWC doses, suggesting a threshold beyond which those benefits diminish. In vineyard systems, Pinamonti (1998) found that MSWC mulch increased SOM, available P, and exchangeable K, confirming its role as an effective substitute for mineral fertilizers. Abbey et al. (2021b) showed that MSWC significantly improved soil fertility when compared to untreated plots. Their study noted that MSWC-amended soils exhibited higher levels of SOM, macro and micronutrients, and CEC.

Finally, improvements in biological fertility and soil functional quality were also evident. Morra et al. (2010) explored the dynamics of C stabilization, indicating that organic inputs fostered more favorable C balances in the soil. Similarly, Hemmat et al. (2010) highlighted how increased TOC under MSWC treatments supported better physical conditions for root growth, indirectly enhancing microbial activity and nutrient cycling. No adverse effects on biological fertility were reported following MSWC application in the reviewed literature. However, this may also be a consequence of the limited number of studies specifically addressing this aspect.

## 4.6 Discussion

The aim of this review was to evaluate the scientific literature on the application of MSWC in agriculture, focusing on its impact on crop productivity and soil fertility, and to discuss its implications for sustainable waste recycling strategies.

To orient the reader, the principal results are synthesized in the summary figures: a dose-response map of average yield versus MSWC dose across cropping systems (Fig. 10) and a trade-off schematic of benefits, risks, and research needs (Fig. 11). Together, these visuals summarize the evidence base on crop productivity and soil fertility and frame the discussion that follows.

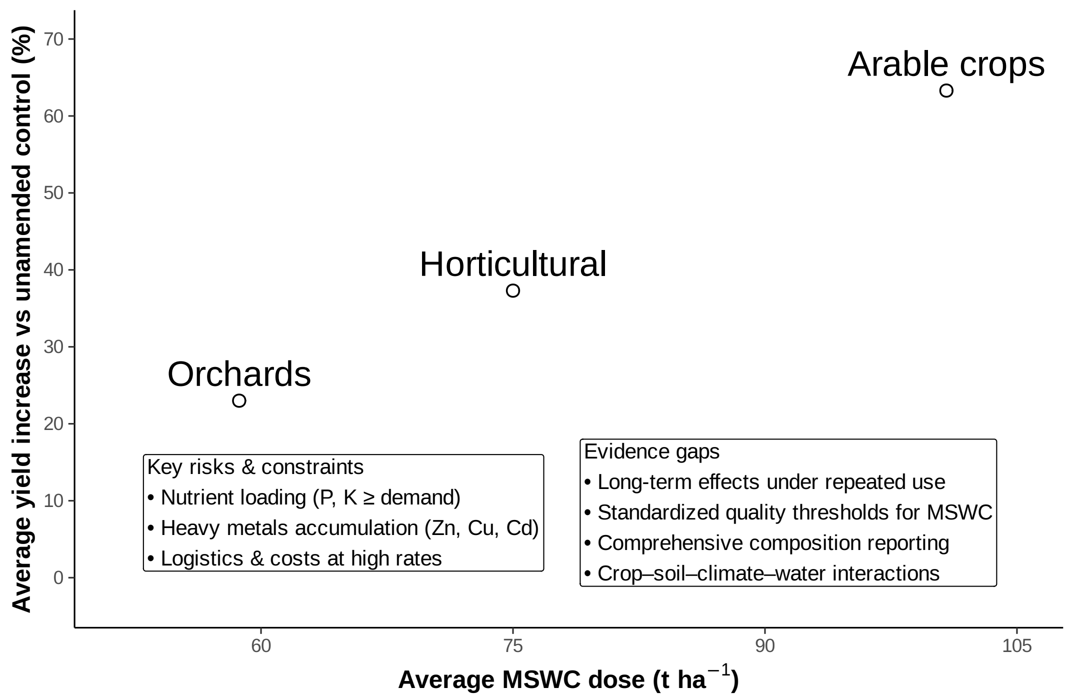


Figure 10: Average crop yield increase as a function of MSWC dose (t ha$^{-1}$, dry matter basis) across arable, horticultural, and orchard systems. Values represent mean responses relative to unamended controls. Callout boxes summarize key risks and constraints (e.g., nutrient loading when P and K exceed crop demand; heavy-metal accumulation; logistics at high rates) and evidence gaps (e.g., long-term cumulative effects, standardized quality thresholds, incomplete composition reporting, crop–soil–climate–water interactions). Abbreviation: MSWC = municipal solid-waste compost.

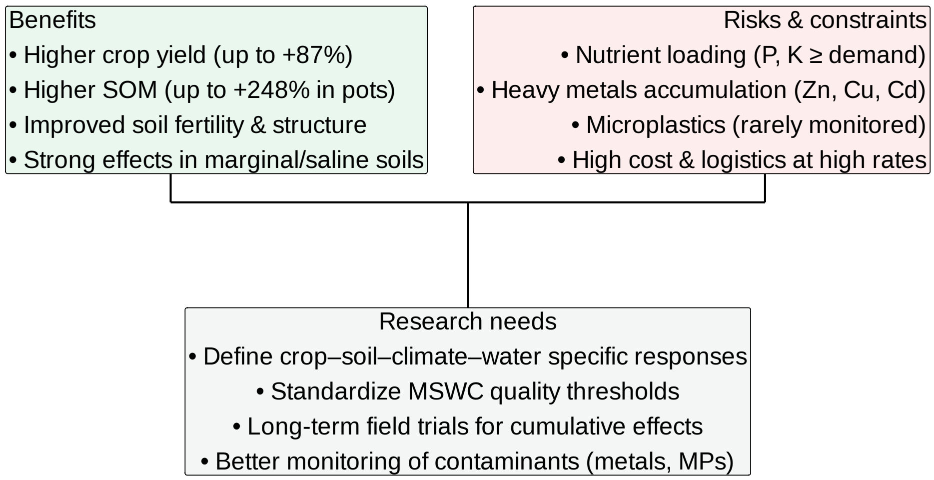


Figure 11: Conceptual summary of the trade-offs associated with MSWC application. Benefits include increased crop yield (up to +87%), higher soil organic matter (SOM; up to +248% in pot trials), improved soil fertility and structure, and positive effects in marginal or saline soils. Risks and constraints include nutrient loading (P and K exceeding crop demand), heavy-metal accumulation (Zn, Cu, Cd), microplastics (rarely monitored), and high costs/logistics at large application rates. Research needs emphasize crop–soil–climate–water specific responses, standardized MSWC quality thresholds, long-term field trials, and improved monitoring of contaminants. Abbreviations: MSWC = municipal solid-waste compost; SOM = soil organic matter; MPs = microplastics.

The response to MSWC application is influenced by several interacting factors, yet some facts emerge clearly across the literature. One of the most frequent and indisputable findings concerns the effectiveness of MSWC when applied at high rates. Studies conducted by different authors reported positive results against unamended plots, indicated with percentage variations from now on, with grain yields increasing up to 87%, when MSWC was applied at rates as high as 80 t ha$^{-1}$ (e.g Lakhdar et al. 2009, 2010; Martinez et al. 2021). Similarly, in studies on horticultural crops, high doses of MSWC (up to 134 t ha$^{-1}$) produced better yields (up to 28%). The highest average application rates were recorded in arable cropping systems (100.8 t ha$^{-1}$), followed by horticultural crops (75 t ha$^{-1}$) and orchard systems (58.7 t ha-$^{1}$). This gradient in applied doses is mirrored by the corresponding average increase in yields, with arable crops showing the greatest improvements (+63.3%), followed by horticultural crops (+37.3%) and orchards (+23%). The positive effect of MSWC application on crop productivity and soil fertility is more pronounced in pot experiments. This enhanced response can be largely attributed to the higher MSWC application rates typically used in such experiments, where, due to logistical simplicity and ease of handling, doses range from a minimum of 3.9 t $ha^{-1}$ to a maximum of 875 t $ha^{-1}$ and are on average 116.1% higher than those applied in field experiments,. As a result, pot studies report significantly greater yield increases (+63.3%) and +248,1% in SOM content when field conditions were able only to reach +2.47% only. In their review Diacono and Montemurro (2010), also emphasized that the best agronomic performances of MSWC are generally achieved with high application rates and frequencies.

Beside the undisputable results for high doses reported above, it can also be reasonably assumed that MSWC positive effects are strongly influenced by pedoclimatic conditions. In adverse soils such as saline-sodic or marginal environments, MSWC has demonstrated a remarkable efficacin enhancing both crop yields and overall soil health. For example, Zaman et al. (2020) found that MSWC not only increased yields (+69,7%) of rice and wheat grown in saline-sodic soils, but also improved soil properties by reducing EC down to -38,8% and increasing SOM up to 45%. Similarly, notable benefits have been observed in semiarid shrublands and marginal soils, where MSWC application led to increased biomass production and thicker plant cover, confirming the positive role of MSWC in environments with low natural fertility and limited water availability (Cuevas et al. 2000; Zając and Skrajna 2024). These findings suggest that MSWC application strategies should be tailored to the local environmental context to optimize agronomic outcomes (Mbarki et al. 2022; Meena et al. 2022). The reliance on high MSWC application rates presents also several concerns. The distribution of large quantities of compost may not be feasible or cost-effective, particularly for farmers in regions with limited compost availability or high transportation costs. From a nutrient-loading perspective, across reviewed field studies, MSWC supplied on average 485 kg N, 140 kg P, and 218 kg K $ha^{-1}$ $yr^{-1}$ (total basis; TableS11 ). Assuming conservative first-year availability (N 10-20%, P 50%, K 80%), this corresponds to ~50–100 kg N, ~70 kg P (~160 kg $P_2O_5$), and ~175 kg K (~210 kg $K_2O$) $ha^{-1}$ $yr^{-1}$. While N availability from MSWC typically does not exceed crop N demand, P and K inputs frequently meet or exceed typical requirements-especially in arable and perennial systems-indicating a risk of P accumulation and K-related cation imbalances under repeated high rates.

Those elevated application rates could also favour the accumulation of heavy metals in soils, further emphasizing a rigorous selection in waste collection and composting procedures to ensure high quality MSWC. Elements, such as Zn, Cu, and Cd, present in MSWC can pose serious treats to soil health and food safety. As reported by Abbey et al. (2021a), MSWC application led to significant increases in soil concentrations of these metals. Metal accumulation can occur even at moderate rates, leading to increased salinity and potential toxicity (Bhardwaj et al. 2023; Mbarki et al. 2008). Additionally, even when total heavy metal content remains below legal limits, repeated applications may lead to long-term build-up in soils. However, limited research has thoroughly investigated the extent of heavy metal contamination in compost or its long-term consequences on soil ecosystems and crop health (Alashty et al. 2011).

Although the facts described so far are well established, some aspects have not yet been fully clarified. The agronomic response to MSWC is context-dependent, arising from interactions among application rate, MSWC type/composition, baseline soil properties (e.g., texture, salinity, fertility), climate/season, and water management, in addition to crop identity (Horrocks et al. 2014; Jodar et al. 2017; Shiralipour et al. 1992). Although numerous studies have demonstrated the MSWC potential to boost crop productivity, these impacts are not consistently observed across all crop types and pedoclimatic settings. Cereal crops, such as maize and wheat, have exhibited notable yield increases under MSWC treatment, with improvements reaching up to 85% (Prabpai et al. 2009; Zaman et al. 2020). Other cereals, like triticale, did not show significant yield benefits under MSWC application, highlighting that not all crops respond positively to it (Kchaou et al. 2018). Additionally, in the context of tobacco grown under deficit irrigation, yield reductions were observed despite improvements in soil water retention and NUE, suggesting that physiological crop responses may override soil-related benefits (Sifola et al. 2025). Crop-specific responses were also reported over a decade ago by Diacono and Montemurro (2010). Overall, this variability argues for tailoring MSWC management to the crop–soil–climate–water context; although spring–summer horticultural species often show larger responses under comparable rates and materials (Zaccardelli et al. 2021), attributing differences to crop type alone is unwarranted without meta-analytic separation of effects.

The variability in MSWC composition remains quite uncertain in the reviewed literature and is an issued that still shows gaps,. The physical, chemical and biological composition of MSWC is highly variable due to the diverse nature of the waste streams from which it is derived. This variability can influence the concentrations of both beneficial nutrients and harmful contaminants, including heavy metals and, microplastics (MPs) (Vithanage et al. 2021). The nutrient and contaminant profile of the MSWC may differ significantly depending on where, when and how the raw materials were collected and processed. Such variability complicates the ability to reliably predict the agronomic benefits of compost and to accurately assess its environmental safety (Miyamoto et al. 2023). Consistently with this, across the studies reporting composition (Table S10), pH ranged from 6.3 to 9.5 (mean 7.8), EC from 0.4 to 23.2 dS m-1 (mean 6.6), total C from 8.7% to 89.3% d.m. (mean 23.3%), and total N from 0.51% to 4.97% d.m. (mean 1.7%). Additionally, many studies do not provide a full physico-chemical characterization of the compost used, especially regarding heavy metals, pathogen load, or microplastic content. Among the 115 articles reviewed, total C was reported in 77 studies (ranging between 8.7 and 89.3% d.m.), total N in 90 (0.51 - 4.97%), and pH in 74 (6.3 - 9.5), while heavy metals were reported in 66 studies (57.4%) and only 1 study reported pathogens and 1 microplastics (see Supplementary Tables S10 - S15). This uneven coverage limits comparability and reduces reproducibility and transferability across regions and systems.

The introduction of MPs into MSWC represents the most recent critical issue. The ubiquity of MPs in urban environments, can readily make their way into MSWC (Vithanage et al. 2021). While current European regulations do not explicitly address MPs in compost, the Italian legislation sets a threshold $\geq$ 2 mm to exclude plastic fragments from MSWC, as defined in the Ministerial Decree of 2 February 2022 (Ministero delle Politiche Agricole Alimentari e Forestali 2022). However, this size is well above the typical dimensions of MPs, which are generally lower than 1 mm, highlighting a regulatory gap in controlling their presence. Once these MPs are incorporated into soils, they may persist for an extended period of time and potentially disrupt soil structure, water retention capabilities, and microbial community dynamics (Kumar et al. 2020; Steiner et al. 2022). The growing recognition of MPs' pollution is an emerging environmental concern, therefore the studies reviewed here do not adequately address this issue, constituting a significant gap in the current research.

In addition to the limitations of the evidence, some constraints related to the review process itself should be acknowledged. The review was restricted to peer-reviewed articles written in English and indexed in the Web of Science, which may have led to the exclusion of relevant grey literature or studies published in other languages or databases. While the use of Latent Dirichlet Allocation helped to refine the literature selection in an objective and reproducible manner, some potentially relevant studies may have been missed due to their limited representation within the extracted topics. Moreover, although each article was manually screened to ensure clarity of objectives and quantitative outcomes, the absence of a formal risk of bias assessment limits the ability to systematically evaluate methodological quality. The heterogeneity of study designs and reporting formats also prevented a quantitative meta-analysis, reducing the extent to which results can be generalized.

Finally, this review outlines clear research priorities to improve the effective and safe use of

MSWC in different farming systems. Identifying crop-specific and pedoclimatic-specific responses to MSWC is essential to optimize its use and to guide farmers toward best practices tailored to local conditions. At the same time, research should seek to define optimal trade-offs between compost application rates, agronomic efficacy, economic viability, and environmental safety. In this regard, future efforts should also focus on establishing the optimal balance between application rates, cost-effectiveness, and environmental sustainability across a wide range of agricultural systems. Particular attention should be paid to the long-term implications of MSWC use, especially in relation to heavy metal accumulation and the fate of MPs in agroecosystems, which have yet to be adequately characterized. These concerns call for standardized methodologies to monitor compost quality and for the establishment of clear safety thresholds to prevent cumulative contamination. .Therefore, some standardization and rigorous quality control measures are essential to ensure that compost consistently meets agricultural and environmental standards and it does not introduce harmful substances into the soil. Lastly, long-term field studies are necessary to fully capture the dynamics of MSWC in soil systems over multiple cropping cycles, particularly its contribution to soil structure, organic matter stabilization, and sustained productivity in diverse farming systems.

## 4.7 Conclusions

The use of Municipal Solid Waste Compost (MSWC) in agriculture offers an alternative strategy to enhance crop yields and soil fertility, while contributing to waste recycling and circular economy goals. However, evidence from the literature spanning from 1985 to 2024 indicates that the effectiveness of MSWC is particularly pronounced at application rates typically higher than 50 t $ha^{-1}$. Conversely, lower doses give more inconsistent results, with some studies reporting beneficial effects, while others highlighting limited or uncertain outcomes. Across studies, positive effects are most consistently observed at medium-high application rates, whereas responses at lower rates are often smaller or inconsistent; however, the economic and logistical feasibility of high-dose applications remains a concern, particularly in large-scale field settings. Crop response varies substantially across species and pedoclimatic conditions, suggesting that the benefits given by MSWC application are highly context-dependent. Therefore it is challenging to draw univocal conclusions about its performance across diverse agricultural systems. The inherent variability in the physical, chemical, and biological composition of MSWC is a critical aspect, driven by the heterogeneous nature of urban waste streams and processing practices. This variability affects both the nutrient value and the contaminant load of compost, complicating its predictable and safe integration into farming systems. Nevertheless, some findings can provide guidance to optimize its use. However, the risks associated with heavy metals and/or microplastics, suggest a future rigorous monitoring of the MSWC production process and quality control. Further efforts are required to assess and mitigate these risks, ensuring the safe and sustainable use of MSWC in agricultural systems.

Future research should prioritize long-term field trials to better understand the cumulative effects of MSWC on soil health, soil organic matter dynamics, and productivity across different cropping systems. Investigating the role of MSWC in soil C sequestration and ecosystem resilience under climate stress scenarios also represents a valuable research frontier. Ultimately, with continued innovation, regulatory development, and scientific support, MSWC can play a central role in advancing sustainable agriculture, enhancing food security, and closing nutrient cycles in line with circular economy principles.

## 4.8 References

Abbey L, Ijenyo M, Spence B, Asunni AO, Ofoe R, Amo-Larbi V (2021a) Bioaccumulation of chemical elements in vegetables as influenced by application frequency of municipal solid waste compost. Canadian Journal of Plant Science 101:967–983. https://doi.org/10.1139/cjps-2020-0291

Abbey L, Ofoe R, Gunupuru LR, Ijenyo M (2021b) Variation in frequency of CQA-tested municipal solid waste compost can alter metabolites in vegetables. Food Research International 143:110225. https://doi.org/10.1016/j.foodres.2021.110225

Aguilar FJ, González P, Revilla J, De León JJ, Porcel O (1997) Agricultural Use of Municipal Solid Waste on Tree and Bush Crops. Journal of Agricultural Engineering Research 67:73–79. https://doi.org/10.1006/jaer.1997.0149

Alashty S, Bahmanyar M, Sepanlou M (2011) Change of pH, organic carbon (OC), electrical conductivity (EC), nickel (Ni) and chrome (Cr) in soil and concentration of Ni and Cr in radish and lettuce plants as influenced by three year application of municipal compost. African journal of agricultural research 6:3740–3746. https://doi.org/10.5897/AJAR10.813

Babu R, Prieto Veramendi PM, Rene ER (2021) Strategies for resource recovery from the organic fraction of municipal solid waste. Case Studies in Chemical and Environmental Engineering 3:100098. https://doi.org/10.1016/j.cscee.2021.100098

Baldi E, Marcolini G, Quartieri M, Sorrenti G, Toselli M (2014) Effect of organic fertilization on nutrient concentration and accumulation in nectarine (Prunus persica var. Nucipersica) trees: The effect of rate of application. Scientia Horticulturae 179:174–179. https://doi.org/10.1016/j.scienta.2014.09.029

Baldi E, Toselli M, Marcolini G, Quartieri M, Cirillo E, Innocenti A, Marangoni B (2010) Compost can successfully replace mineral fertilizers in the nutrient management of commercial peach orchard. Soil Use and Management 26:346–353. https://doi.org/10.1111/j.1475-2743.2010.00286.x

Barles S (2014) History of Waste Management and the Social and Cultural Representations of Waste. In: Agnoletti M, Neri Serneri S (eds) The Basic Environmental History. Springer International Publishing, Cham, pp 199–226

Basso-Figuera C, Schaffer B, Crane JH, Colls AM, Bryan HH (1995) Mineral nutrient content, growth, and yield of papaya (Carica papaya L.) as influenced by trench depth and municipal solid waste compost. Mineral nutrient content, growth, and yield of papaya (Carica papaya L) as influenced by trench depth and municipal solid waste compost 72:231–235

Belén Almendro-Candel M, Navarro-Pedreño J, Gómez Lucas I, A. Zorpas A, Voukkali I, Loizia

P (2019) The Use of Composted Municipal Solid Waste under the Concept of Circular Economy and as a Source of Plant Nutrients and Pollutants. In: El-Din Mostafa Saleh H (ed) Municipal Solid Waste Management. IntechOpen

Bhardwaj P, Sharma RK, Chauhan A, Ranjan A, Rajput VD, Minkina T, Mandzhieva SS, Mina U, Wadhwa S, Bobde P, Tripathi A (2023) Assessment of Heavy Metal Distribution and Health Risk of Vegetable Crops Grown on Soils Amended with Municipal Solid Waste Compost for Sustainable Urban Agriculture. Water 15:228. https://doi.org/10.3390/w15020228

Blanco I, Cardinale M, Domanda C, Pappaccogli G, Romano P, Zorzi G, Rustioni L (2024) Mulching with Municipal Solid Waste (MSW) Compost Has Beneficial Side Effects on Vineyard Soil Compared to Mulching with Synthetic Films. Horticulturae 10:769. https://doi.org/10.3390/horticulturae10070769

Bona D, Cristoforetti A, Zanzotti R, Bertoldi D, Dellai N, Silvestri S (2022) Matured Manure and Compost from the Organic Fraction of Solid Waste Digestate Application in Intensive Apple Orchards. International Journal of Environmental Research and Public Health 19:15512. https://doi.org/10.3390/ijerph192315512

Cao X, Williams PN, Zhan Y, Coughlin SA, McGrath JW, Chin JP, Xu Y (2023) Municipal solid waste compost: Global trends and biogeochemical cycling. Soil & Environmental Health 1:100038. https://doi.org/10.1016/j.seh.2023.100038

Castaldi S, Bertolini T, Vannini A, Marinari S, Chilosi G (2023) Effect of Compost Derived from Urban Waste on Chard (Beta vulgaris L., var cycla) Yield and Soil GHG Fluxes in a Mediterranean Agricultural System. Atmosphere 14:246. https://doi.org/10.3390/atmos14020246

Cercioglu M (2019) Compost Effects on Soil Nutritional Quality and Pepper (Capsicum annuum L.) Yield. Tarım Bilimleri Dergisi 25:155–162. https://doi.org/10.15832/ankutbd.396547

Chen DM-C, Bodirsky BL, Krueger T, Mishra A, Popp A (2020) The world's growing municipal solid waste: Trends and impacts. Environmental Research Letters 15:074021. https://doi.org/10.1088/1748-9326/ab8659

Clark GA, Stanley CD, Maynard DN (2000) Municipal solid waste compost (MSWC) as a soil amendment in irrigated vegetable production. Transactions of the ASAE 43:847–853. https://doi.org/10.13031/2013.2979

Coria-Cayupán YS, Sánchez de Pinto MI, Nazareno MA (2009) Variations in Bioactive Substance Contents and Crop Yields of Lettuce (*Lactuca Sativa* L.) Cultivated in Soils with Different Fertilization Treatments. Journal of Agricultural and Food Chemistry 57:10122–10129. https://doi.org/10.1021/jf903019d

Cuevas G, Blázquez R, Martinez F, Walter I (2000) Composted MSW Effects on Soil Properties and Native Vegetation in a Degraded Semiarid Shrubland. Compost Science & Utilization 8:303–309. https://doi.org/10.1080/1065657X.2000.10702003

De Nobile FO, Calero Hurtado A, Prado RDM, De Souza HA, Anunciação MG, Palaretti LF, Dezem LASN (2021) A Novel Technology for Processing Urban Waste Compost as a Fast-Releasing Nitrogen Source to Improve Soil Properties and Broccoli and Lettuce Production. Waste and Biomass Valorization 12:6191–6203. https://doi.org/10.1007/s12649-021-01415-z

De Varennes A, Abreu MM, Qu G, Cunha-Queda C (2010) Enzymatic Activity of a Mine Soil Varies According to Vegetation Cover and Level of Compost Applied. International Journal of Phytoremediation 12:371–383. https://doi.org/10.1080/15226510903051757

Diacono M, Montemurro F (2010) Long-term effects of organic amendments on soil fertility. A review. Agronomy for Sustainable Development 30:401–422. https://doi.org/10.1051/agro/2009040

Elbl J, Maková J, Javoreková S, Medo J, Kintl A, Lošák T, Lukas V (2019) Response of Microbial Activities in Soil to Various Organic and Mineral Amendments as an Indicator of Soil Quality. Agronomy 9:485. https://doi.org/10.3390/agronomy9090485

Erana FG, Tenkegna TA, Asfaw SL (2019) Effect of agro industrial wastes compost on soil health and onion yields improvements: Study at field condition. International Journal of Recycling of Organic Waste in Agriculture 8:161–171. https://doi.org/10.1007/s40093-019-0286-2

Fagnano M, Adamo P, Zampella M, Fiorentino N (2011) Environmental and agronomic impact of fertilization with composted organic fraction from municipal solid waste: A case study in the region of Naples, Italy. Agriculture, Ecosystems & Environment 141:100–107. https://doi.org/10.1016/j.agee.2011.02.019

FAO (2018) The 10 Elements of Agroecology. Guiding the Transition to Sustainable Food and Agricultural Systems.

Farneselli M, Pannacci E, Tei F (2022) Does the timing of short-term biowaste compost application affect crop growth and potential nitrate leaching? The case studies of processing tomato and cauliflower under field conditions. Italian Journal of Agronomy 17:2045. https://doi.org/10.4081/ija.2022.2045

Feller C, Blanchart E, Bernoux M, Lal R, Manlay R (2012) Soil fertility concepts over the past two centuries: The importance attributed to soil organic matter in developed and developing countries. Archives of Agronomy and Soil Science 58:S3–S21. https://doi.org/10.1080/03650340.2012.693598

Fellows I, Fellows MI, Rcpp L, Rcpp L (2018) Package “wordcloud.” R package version 2:331

Gadaleta A, Lacolla G, Giove SL, Fortunato S, Nigro D, Mastro MA, De Corato U, Caranfa D, Cucci G, De Pinto MC, Vita F (2022) Durum Wheat Response to Organic and Mineral Fertilization with Application of Different Levels and Types of Phosphorus-Based Fertilizers. Agronomy 12:1861. https://doi.org/10.3390/agronomy12081861

Gallardo-Lara F, Azcón M, Polo A (2006) Phytoavailability and Fractions of Iron and Manganese in Calcareous Soil Amended with Composted Urban Wastes. Journal of Environmental Science and Health, Part B 41:1187–1201. https://doi.org/10.1080/03601230600856991

Garrett RD, Ryschawy J, Bell LW, Cortner O, Ferreira J, Garik AVN, Gil JDB, Klerkx L, Moraine M, Peterson CA, Dos Reis JC, Valentim JF (2020) Drivers of decoupling and recoupling of crop and livestock systems at farm and territorial scales. E&S 25:art24. https://doi.org/10.5751/ES-11412-250124

Golabi MH, Galsim FP, Endale D, Tareyama SA, Iyekar C (2021) Agronomic Value of Composted Organic Waste Application on Porous Soils of Northern Guam. Malaysian Journal of Soil Science 25:

Gómez-Muñoz B, Magid J, Jensen LS (2017) Nitrogen turnover, crop use efficiency and soil fertility in a long-term field experiment amended with different qualities of urban and agricultural waste. Agriculture, Ecosystems & Environment 240:300–313. https://doi.org/10.1016/j.agee.2017.01.030

Grün B, Hornik K (2011) Topicmodels: An R Package for Fitting Topic Models. Journal of Statistical Software 40:1–30. https://doi.org/10.18637/jss.v040.i13

Guerini G, Maffeis P, Allievi L, Gigliotti C (2006) Integrated Waste Management in a Zone of Northern Italy: Compost Production and Use, and Analytical Control of Compost, Soil, and Crop. Journal of Environmental Science and Health, Part B 41:1203–1219. https://doi.org/10.1080/03601230600857031

Güldner D, Krausmann F (2017) Nutrient recycling and soil fertility management in the course of the industrial transition of traditional, organic agriculture: The case of Bruck estate, 1787–1906. Agriculture, Ecosystems & Environment 249:80–90. https://doi.org/10.1016/j.agee.2017.07.038

Hargreaves J, Adl M, Warman P (2008) A review of the use of composted municipal solid waste in agriculture. Agriculture, Ecosystems & Environment 123:1–14. https://doi.org/10.1016/j.agee.2007.07.004

Hemmat A, Aghilinategh N, Rezainejad Y, Sadeghi M (2010) Long-term impacts of municipal solid waste compost, sewage sludge and farmyard manure application on organic carbon, bulk density and consistency limits of a calcareous soil in central Iran. Soil and Tillage

Research 108:43–50. https://doi.org/10.1016/j.still.2010.03.007

Hodson AK, Sayre JM, Lyra MCCP, Rodrigues JLM (2021) Influence of Recycled Waste Compost on Soil Food Webs, Nutrient Cycling and Tree Growth in a Young Almond Orchard. Agronomy 11:1745. https://doi.org/10.3390/agronomy11091745

Horrocks A, Tregurtha C, Meenken E, Maley S, Beare M (2014) Potential for municipal compost to reduce inorganic fertiliser use in crop production systems. Acta Horticulturae 315–323. https://doi.org/10.17660/ActaHortic.2014.1018.33

Hossain S, Al-Solaimani SGM, Alghabari F, Shahzad K, Rashid MI (2024) Enhancing maize yield through sustainable and eco-friendly practices: The impact of municipal organic waste compost and soil amendments. Cogent Food & Agriculture 10:2307119. https://doi.org/10.1080/23311932.2024.2307119

Jelodar H, Wang Y, Yuan C, Feng X, Jiang X, Li Y, Zhao L (2019) Latent Dirichlet Allocation (LDA) and topic modeling: Models, applications, a survey. Multimedia Tools and Applications 78:15169–15211. https://doi.org/10.1007/s11042-018-6894-4

Jodar JR, Ramos N, Carreira JA, Pacheco R, Fernández-Hernández A (2017) Quality assessment of compost prepared with municipal solid waste. Open Engineering 7:221–227. https://doi.org/10.1515/eng-2017-0028

Jorge-Mardomingo I, Jiménez-Hernández ME, Moreno L, De La Losa A, De La Cruz MT, Casermeiro MÁ (2015) Application of high doses of organic amendments in a Mediterranean agricultural soil: An approach for assessing the risk of groundwater contamination. CATENA 131:74–83. https://doi.org/10.1016/j.catena.2015.03.013

Jorge-Mardomingo I, Soler-Rovira P, Casermeiro MÁ, De La Cruz MT, Polo A (2013) Seasonal changes in microbial activity in a semiarid soil after application of a high dose of different organic amendments. Geoderma 206:40–48. https://doi.org/10.1016/j.geoderma.2013.04.025

Kasthuri H, Shanthi K, Sivakumar S, Rajakumar S, Son H, Song Y (2011) Influence of municipal solid waste compost (MSWC) on the growth and yield of green gram (Vigna radiate (L) Wilczek), Fenugreek (Trigonella foenum-graecum L.) and on soil quality. Journal of Environmental Health Science & Engineering 8:280–285

Kaza S, Yao LC, Bhada-Tata P, Van Woerden F (2018) What a Waste 2.0: A Global Snapshot of Solid Waste Management to 2050. Washington, DC: World Bank

Kchaou R, Baccar R, Bouzid J, Rejeb S (2018) The impact of sewage sludge and compost on winter triticale. Environmental Science and Pollution Research 25:18314–18319. https://doi.org/10.1007/s11356-017-0609-7

Kumar M, Xiong X, He M, Tsang DCW, Gupta J, Khan E, Harrad S, Hou D, Ok YS, Bolan NS

(2020) Microplastics as pollutants in agricultural soils. Environmental Pollution 265:114980. https://doi.org/10.1016/j.envpol.2020.114980

Lakhdar A, Achiba WB, Montemurro F, Jedidi N, Abdelly C (2009) Effect of Municipal Solid Waste Compost and Farmyard Manure Application on Heavy-Metal Uptake in Wheat. Communications in Soil Science and Plant Analysis 40:3524–3538. https://doi.org/10.1080/00103620903326040

Lakhdar A, Falleh H, Ouni Y, Oueslati S, Debez A, Ksouri R, Abdelly C (2011) Municipal solid waste compost application improves productivity, polyphenol content, and antioxidant capacity of Mesembryanthemum edule. Journal of Hazardous Materials 191:373–379. https://doi.org/10.1016/j.jhazmat.2011.04.092

Lakhdar A, Iannelli MA, Debez A, Massacci A, Jedidi N, Abdelly C (2010) Effect of municipal solid waste compost and sewage sludge use on wheat ( *Triticum Durum* ): Growth, heavy metal accumulation, and antioxidant activity. Journal of the Science of Food and Agriculture 90:965–971. https://doi.org/10.1002/jsfa.3904

Leogrande R, Lopedota O, Fiore A, Vitti C, Ventrella D, Montemurro F (2013) Previous crops and organic fertilizers in lettuce: Effects on yields and soil properties. Journal of Plant Nutrition 36:1945–1962. https://doi.org/10.1080/01904167.2012.754042

Leogrande R, Lopedota O, Vitti C, Ventrella D, Montemurro F (2014) Effects of irrigation volumes and organic fertilizers on eggplant grown in Mediterranean environment. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science 64:518–528. https://doi.org/10.1080/09064710.2014.927526

Leogrande R, Lopedota O, Vitti C, Ventrella D, Montemurro F (2016a) Saline water and municipal solid waste compost application on tomato crop: Effects on plant and soil. Journal of Plant Nutrition 39:491–501. https://doi.org/10.1080/01904167.2015.1084325

Leogrande R, Vitti C, Lopedota O, Ventrella D, Montemurro F (2016b) Saline water and MSW compost: Effects on yield of maize crop and soil responses. Journal of Plant Nutrition 39:1863–1873. https://doi.org/10.1080/01904167.2016.1187744

Lou XF, Nair J (2009) The impact of landfilling and composting on greenhouse gas emissions – A review. Bioresource Technology 100:3792–3798. https://doi.org/10.1016/j.biortech.2008.12.006

Manea EE, Bumbac C, Dinu LR, Bumbac M, Nicolescu CM (2024) Composting as a Sustainable Solution for Organic Solid Waste Management: Current Practices and Potential Improvements. Sustainability 16:6329. https://doi.org/10.3390/su16156329

Manlay RJ, Feller C, Swift MJ (2007) Historical evolution of soil organic matter concepts and their relationships with the fertility and sustainability of cropping systems. Agriculture, Ecosystems & Environment 119:217–233. https://doi.org/10.1016/j.agee.2006.07.011

Martinez S, Gabriel J, Allende-Montalbán R, San-Juan-Heras R, Delgado M (2022) The Application of a Bio-Stabilized Municipal Solid Waste-Based Fertilizer for Buckwheat Production. Agriculture 12:776. https://doi.org/10.3390/agriculture12060776

Martinez S, Sánchez-Moreno S, Gabriel JL, Álvarez C, Delgado MDM (2021) Valorization of a Bio-Stabilized Municipal Solid Waste Amendment for Faba Bean (Vicia faba L.) Fertilization. Agriculture 11:1109. https://doi.org/10.3390/agriculture11111109

Martínez-Blanco J, Lazcano C, Christensen TH, Muñoz P, Rieradevall J, Møller J, Antón A, Boldrin A (2013) Compost benefits for agriculture evaluated by life cycle assessment. A review. Agronomy for Sustainable Development 33:721–732. https://doi.org/10.1007/s13593-013-0148-7

Mbarki S, Labidi N, Mahmoudi H, Jedidi N, Abdelly C (2008) Contrasting effects of municipal compost on alfalfa growth in clay and in sandy soils: N, P, K, content and heavy metal toxicity. Bioresource Technology 99:6745–6750. https://doi.org/10.1016/j.biortech.2008.01.010

Mbarki S, Skalicky M, Talbi O, Chakraborty A, Hnilicka F, Hejnak V, Zivcak M, Brestic M, Cerda A, Abdelly C (2020) Performance of *Medicago Sativa* Grown in Clay Soil Favored by Compost or Farmyard Manure to Mitigate Salt Stress. Agronomy 10:94. https://doi.org/10.3390/agronomy10010094

Mbarki S, Talbi O, Skalicky M, Vachova P, Hejnak V, Hnilicka F, Al-ashkar I, Abdelly C, Rahman MA, El Sabagh A, Tlustos P (2022) Comparison of grain sorghum and alfalfa for providing heavy metal remediation of sandy soil with different soil amendments and salt stress. Frontiers in Environmental Science 10:1022629. https://doi.org/10.3389/fenvs.2022.1022629

Meena MD, Narjary B, Sheoran P, Jat HS, Joshi PK, Chinchmalatpure AR (2022) Changes in physical and chemical properties of saline soil amended with municipal solid waste compost and chemical fertilizers in a mustard–pearl millet cropping system. Land Degradation & Development 33:1677–1688. https://doi.org/10.1002/ldr.4256

Mehdi SM, Sarfraz M, Ilyas M, Amjad Qureshi M, Zaka MA (2015) Integrated Nutrient Management using P-fixation Factor in Rice-wheat Cropping System under Salt Affected Conditions. International Journal of Agriculture and Biology 17:643–647. https://doi.org/10.17957/IJAB/17.3.13.930

Ministero delle Politiche Agricole Alimentari e Forestali (2022) Decreto 2 febbraio 2022: Aggiornamento degli allegati 2 e 7 al decreto legislativo 29 aprile 2010, n. 75

Misra RV, Roy RN, Hiraoka H (2003) On-farm composting methods. Rome, Italy: UN-FAO

Miyamoto H, Shigeta K, Suda W, et al (2023) An agroecological structure model of compost—soil—plant interactions for sustainable organic farming. ISME Communications 3:28. https://doi.org/10.1038/s43705-023-00233-9

Montemurro F (2009) Different Nitrogen Fertilization Sources, Soil Tillage, and Crop Rotations in Winter Wheat: Effect on Yield, Quality, and Nitrogen Utilization. Journal of Plant Nutrition 32:1–18. https://doi.org/10.1080/01904160802530979

Montemurro F, Charfeddine M, Maiorana M, Convertini G (2010) Compost Use in Agriculture: The Fate of Heavy Metals in Soil and Fodder Crop Plants. Compost Science & Utilization 18:47–54. https://doi.org/10.1080/1065657X.2010.10736933

Montemurro F, Maiorana M, Convertini G, Ferri D (2006) Compost Organic Amendments in Fodder Crops: Effects on Yield, Nitrogen Utilization and Soil Characteristics. Compost Science & Utilization 14:114–123. https://doi.org/10.1080/1065657X.2006.10702272

Montemurro F, Maiorana M, Convertini G, Ferri D (2007) Alternative sugar beet production using shallow tillage and municipal solid waste fertiliser. Agronomy for Sustainable Development 27:129–137. https://doi.org/10.1051/agro:2006032

Montemurro F, Maiorana M, Convertini G, Fornaro F (2005) Improvement of soil properties and nitrogen utilisation of sunflower by amending municipal solid waste compost. Agronomy for Sustainable Development 25:369–375. https://doi.org/10.1051/agro:2005038

Moraes MTD, Silva VRD, Cherubin MR, Carlesso R, Debiasi H, Levien R (2014) Changes in a Rhodic Hapludox under no-tillage and urban waste compost in the northwest of Rio Grande do Sul, Brazil. Revista Brasileira de Ciência do Solo 38:1327–1336. https://doi.org/10.1590/S0100-06832014000400028

Moretti B, Bertora C, Grignani C, Lerda C, Celi L, Sacco D (2020) Conversion from mineral fertilisation to MSW compost use: Nitrogen fertiliser value in continuous maize and test on crop rotation. Science of The Total Environment 705:135308. https://doi.org/10.1016/j.scitotenv.2019.135308

Morra L, Pagano L, Iovieno P, Baldantoni D, Alfani A (2010) Soil and vegetable crop response to addition of different levels of municipal waste compost under Mediterranean greenhouse conditions. Agronomy for Sustainable Development 30:701–709. https://doi.org/10.1051/agro/2009046

Mousavi SM, Bahmanyar MA, Pirdashti H, Moradi S (2017) Nutritional (Fe, Mn, Ni, and Cr) and growth responses of rice plant affected by perennial application of two bio-solids. Environmental Monitoring and Assessment 189:340. https://doi.org/10.1007/s10661-017-6050-z

Mylavarapu RS, Zinati GM (2009) Improvement of soil properties using compost for optimum parsley production in sandy soils. Scientia Horticulturae 120:426–430. https://doi.org/10.1016/j.scienta.2008.11.038

Novinscak A, Goyer C, Wilson C, Zebarth BJ, Burton DL, Chantigny MH, Filion M (2022) Seasonal changes in the abundance and activity of bacterial and fungal denitrifying communities associated with different compost amendments. Canadian Journal of Microbiology 68:91–102. https://doi.org/10.1139/cjm-2021-0256

Oguntade OA, Olagbenro TS, Odusanya OA, Olagunju SO, Adewusi KM, Adegoke AT (2019) Assessment of composted kitchen waste and poultry manure amendments on growth, yield and heavy metal uptake by Jute mallow Corchorus olitorius Linn. International Journal of Recycling of Organic Waste in Agriculture 8:187–195. https://doi.org/10.1007/s40093-018-0232-8

Okoli F, Chukwura E, Mbachu A (2022) Quality assessment of compost produced from agricultural wastes: Impact on the growth and yield of *Zea Mays L.* International journal of recycling organic waste in agriculture. https://doi.org/10.30486/ijrowa.2022.1954860.1430

Ouni Y, Mateos-Naranjo E, Lakhdar A, Andrades-Moreno L, Abdelly C, Barhoumi Z (2014) Municipal Solid Waste Compost Application Improves the Negative Impact of Saline Soil in Two Forage Species. Communications in Soil Science and Plant Analysis 45:1421–1434. https://doi.org/10.1080/00103624.2013.875209

Ozdemir S (2005) Effects of municipal solid waste(MSW) compost on nodulation, plant growth and mineral composition of chickpea in marginal land. Fresenius Environmental Bulletin 14:599–604

Ozores-Hampton M, Bryan HH, Schaffer B, Hanlon EA (1994) Nutrient Concentrations, Growth, and Yield of Tomato and Squash in Municipal Solid-waste-amended Soil. HortScience 29:785–788. https://doi.org/10.21273/HORTSCI.29.7.785

Page MJ, McKenzie JE, Bossuyt PM, et al (2021) The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ n71. https://doi.org/10.1136/bmj.n71

Papafilippaki A, Paranychianakis N, Nikolaidis NP (2015) Effects of soil type and municipal solid waste compost as soil amendment on Cichorium spinosum (spiny chicory) growth. Scientia Horticulturae 195:195–205. https://doi.org/10.1016/j.scienta.2015.09.030

Peltre C, Nyord T, Bruun S, Jensen LS, Magid J (2015) Repeated soil application of organic waste amendments reduces draught force and fuel consumption for soil tillage. Agriculture, Ecosystems & Environment 211:94–101. https://doi.org/10.1016/j.agee.2015.06.004

Pinamonti F (1998) Compost mulch effects on soil fertility, nutritional status and performance of grapevine. Nutrient Cycling in Agroecosystems 51:239–248. https://doi.org/10.1023/A:1009701323580

Prabpai S, Charerntanyarak L, Siri B, Moore MR, Noller BN (2009) Effects of residues from municipal solid waste landfill on corn yield and heavy metal content. Waste Management 29:2316–2320. https://doi.org/10.1016/j.wasman.2009.02.009

Raji M-R, Hassankhah A, Aghaei S, Aalifar M (2016) Influence of Cheaper Plant Media, Municipal Solid Waste Compost (MSWC) and Rice Hulls on Growth Characteristics of Pepper ( *Capsicum Annuum* L.) Seedlings. Journal of Essential Oil Bearing Plants 19:954–962. https://doi.org/10.1080/0972060X.2014.1002013

Rashtbari M, Hossein Ali A, Ghorchiani M (2020) Effect of Vermicompost and Municipal Solid Waste Compost on Growth and Yield of Canola under Drought Stress Conditions. Communications in Soil Science and Plant Analysis 51:2215–2222. https://doi.org/10.1080/00103624.2020.1820023

Razza F, D'Avino L, L'Abate G, Lazzeri L (2018) The Role of Compost in Bio-waste Management and Circular Economy. In: Benetto E, Gericke K, Guiton M (eds) Designing Sustainable Technologies, Products and Policies. Springer International Publishing, Cham, pp 133–143

Rupasinghe ISU, Leelamanie DAL (2020) Comparison of municipal and agriculture-based solid waste composts: Short-term crop-yield response and soil properties in a tropical Ultisol. Biologia 75:809–818. https://doi.org/10.2478/s11756-020-00464-4

Salis L, Cabiddu A, Sanna F, Sitzia M, Carboni G (2024) Municipal solid waste compost use can improve crop barley production and enhance soil chemical fertility. European Journal of Agronomy 153:127064. https://doi.org/10.1016/j.eja.2023.127064

Sánchez-Monedero MA, Cayuela ML, Sánchez-García M, Vandecasteele B, D'Hose T, López G, Martínez-Gaitán C, Kuikman PJ, Sinicco T, Mondini C (2019) Agronomic Evaluation of Biochar, Compost and Biochar-Blended Compost across Different Cropping Systems: Perspective from the European Project FERTIPLUS. Agronomy 9:225. https://doi.org/10.3390/agronomy9050225

Sayğı H (2023) Effect of Municipal Solid Waste Compost on Yield, Plant Growth and Nutrient Elements in Strawberry Cultivation. Sustainability 15:9447. https://doi.org/10.3390/su15129447

Schulz DG, Fey R, Ruppenthal V, Malavasi MDM, Malavasi UC (2012) Initial growth of physic nut as a function of sources and doses of organic fertilizers. Ciência e Agrotecnologia 36:615–623. https://doi.org/10.1590/S1413-70542012000600003

Shiralipour A, McConnell DB, Smith WH (1992) Uses and benefits of MSW compost: A review and an assessment. Biomass and Bioenergy 3:267–279. https://doi.org/10.1016/0961-9534(92)90031-K

Sifola MI, Carrino L, Cozzolino E, Palladino M, Sicignano M, Todisco D, Del Piano L (2025) The Effect of Eco-Friendly/Sustainable Agricultural Practices (Legume Green Manure and Compost Soil Amendment) on a Tobacco Crop Grown Under Deficit Irrigation. Sustainability 17:769. https://doi.org/10.3390/su17020769

Sifola MI, Cozzolino E, Todisco D, Palladino M, Sicignano M, Del Piano L (2024) Organic Fraction Municipal Solid Waste Compost and Horse Bean Green Manure Improve Sustainability of a Top-Quality Tobacco Cropping System: The Beneficial Effects on Soil and Plants. Sustainability 16:6466. https://doi.org/10.3390/su16156466

Silge J, Robinson D (2016) Tidytext: Text Mining and Analysis Using Tidy Data Principles in R. The Journal of Open Source Software 1:37. https://doi.org/10.21105/joss.00037

Soobhany N, Mohee R, Garg VK (2017) A comparative analysis of composts and vermicomposts derived from municipal solid waste for the growth and yield of green bean (*Phaseolus Vulgaris*). Environmental Science and Pollution Research 24:11228–11239. https://doi.org/10.1007/s11356-017-8774-2

Soumaré M, Tack FMG, Verloo MG (2003) Effects of a municipal solid waste compost and mineral fertilization on plant growth in two tropical agricultural soils of Mali. Bioresource Technology 86:15–20. https://doi.org/10.1016/S0960-8524(02)00133-5

Steiner T, Möller JN, Löder MGJ, Hilbrig F, Laforsch C, Freitag R (2022) Microplastic Contamination of Composts and Liquid Fertilizers from Municipal Biowaste Treatment Plants: Effects of the Operating Conditions. Waste and Biomass Valorization. https://doi.org/10.1007/s12649-022-01870-2

Taheri Soudejani H, Shayannejad M, Kazemian H, Heidarpour M, Rutherford M (2020) Effect of co-composting municipal solid waste with Mg-modified zeolite on soil water balance components using HYDRUS-1D. Computers and Electronics in Agriculture 176:105637. https://doi.org/10.1016/j.compag.2020.105637

Terrile R, Martinez N, Paz N, Brunotto F, Costa M, Budai N, Ruiz C, Rizzi M, Invernizzi M, Scarpeci T, Piacentini RD, Winter K, Tornaghi C (2024) Urban food waste for soil amendment? Analysis and characterisation of waste-based compost for soil fertility management in agroecological horticultural production systems in the city of Rosario, Argentina. Frontiers in Sustainable Food Systems 8:1338451. https://doi.org/10.3389/fsufs.2024.1338451

Testani E, Montemurro F, Ciaccia C, Diacono M (2020) Agroecological practices for organic lettuce: Effects on yield, nitrogen status and nitrogen utilisation efficiency. Biological Agriculture & Horticulture 36:84–95. https://doi.org/10.1080/01448765.2019.1689531

Tzortzakis N, Saridakis C, Chrysargyris A (2020) Treated Wastewater and Fertigation Applied for Greenhouse Tomato Cultivation Grown in Municipal Solid Waste Compost and Soil

Mixtures. Sustainability 12:4287. https://doi.org/10.3390/su12104287

Ulm F, Avelar D, Hobson P, Penha-Lopes G, Dias T, Máguas C, Cruz C (2019) Sustainable urban agriculture using compost and an open-pollinated maize variety. Journal of Cleaner Production 212:622–629. https://doi.org/10.1016/j.jclepro.2018.12.069

United Nations Environment Programme, International Solid Waste Association (2024) Global Waste Management Outlook 2024 - Beyond an age of waste: Turning rubbish into a resource. https://doi.org/10.59117/20.500.11822/44939

Valverde-Orozco V, Gavilanes-Terán I, Idrovo-Novillo J, Ramos- Romero S, Valverde-Quiroz D, Idrovo-Gavilanes J, Paredes C (2024) Approach to the circular economy through agro-livestock waste composting with heat recovery and agricultural use of the resulting compost. Sustainable Chemistry and Pharmacy 41:101730. https://doi.org/10.1016/j.scp.2024.101730

Villena R, Castellanos MT, Cartagena MC, Ribas F, Arce A, Cabello MJ, Requejo MI (2018) Winery distillery waste compost effect on the performance of melon crop under field conditions. Scientia Agricola 75:494–503. https://doi.org/10.1590/1678-992x-2016-0507

Vithanage M, Ramanayaka S, Hasinthara S, Navaratne A (2021) Compost as a carrier for microplastics and plastic-bound toxic metals into agroecosystems. Current Opinion in Environmental Science & Health 24:100297. https://doi.org/10.1016/j.coesh.2021.100297

Warman PR, Rodd AV, Hicklenton P (2009) The effect of MSW compost and fertilizer on extractable soil elements and the growth of winter squash in Nova Scotia. Agriculture, Ecosystems & Environment 133:98–102. https://doi.org/10.1016/j.agee.2009.05.010

Warman PR, Vernon Rodd A, Hicklenton P (2011) The Effect of MSW Compost and Fertilizer on Extractable Soil Elements, Tuber Yield, and Elemental Concentrations in the Plant Tissue of Potato. Potato Research 54:1–11. https://doi.org/10.1007/s11540-010-9167-9

Welbers K, Van Atteveldt W, Benoit K (2017) Text Analysis in R. Communication Methods and Measures 11:245–265. https://doi.org/10.1080/19312458.2017.1387238

Wilton MJ, Karagatzides JD, Tsuji LJS (2020) Fertilizing bush beans with locally made compost in a remote subarctic community. Agrosystems, Geosciences & Environment 3:e20109. https://doi.org/10.1002/agg2.20109

Wolkowski RP (2003) Nitrogen Management Considerations for Landspreading Municipal Solid Waste Compost. Journal of Environmental Quality 32:1844–1850. https://doi.org/10.2134/jeq2003.1844

Zaccardelli M, Pane C, Di Mola I, Ronga D, Mori M (2021) Municipal organic waste compost replaces mineral fertilization in the horticultural cropping systems, reducing the pollution risk. Italian Journal of Agronomy 16: https://doi.org/10.4081/ija.2021.1756

Zając M, Skrajna T (2024) Effect of Composted Organic Waste on Miscanthus sinensis Andersson Yield, Morphological Characteristics and Chlorophyll Fluorescence and Content. Agronomy 14:1672. https://doi.org/10.3390/agronomy14081672

Zaman G, Murtaza B, Imran M, Shahid M, Shah GM, Amjad M, Naeem MA, Mubeen M, Murtaza G (2020) Utilization of Bio-Municipal Solid Waste Improves Saline-Sodic Soils and Crop Productivity in Rice-Wheat. Compost Science & Utilization 28:16–27. https://doi.org/10.1080/1065657X.2019.1709106

Zgallai H, Zoghlami RI, Annabi M, Zarrouk O, Jellali S, Hamdi H (2024) Mitigating soil water deficit using organic waste compost and commercial water retainer: A comparative study under semiarid conditions. Euro-Mediterranean Journal for Environmental Integration 9:377–391. https://doi.org/10.1007/s41207-023-00437-4

## 4.9 Declarations


### 4.9.1 Funding

The authors did not receive support from any organization for the submitted work.


### 4.9.2 Conflicts of interest/Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

### 4.9.3 Availability of data and material

The list of reviewed articles is provided as supplementary material.

### 4.9.4 Code availability

The code used for data management and analysis is available from the corresponding author on reasonable request.

### 4.9.5 Ethics approval

Not applicable.

### 4.9.6 Authors' contributions

Francesco Serafini and Gaio Cesare Pacini conceived the idea for the review. Francesco Serafini and Valentina Bertucci performed the literature search and wrote the first draft of the manuscript. Margherita Santoni and Ottorino-Luca Pantani critically revised the text. All authors read and approved the final version of the manuscript.

### 4.9.7 Consent to participate

Not applicable.

### 4.9.8 Consent for publication

Not applicable.

## 4.10 Supplementary Materials

The attached CSV file ("reviewed_papers.csv") contains the full list of reviewed articles, categorized according to the criteria reported in Table 1. For each article, the average application rate of MSWC is provided in t $ha^{-1}$ $y^{-1}$. When the original data were expressed in volume, conversion to mass units was performed assuming an application depth of 0.2 m, a bulk density of 1.3 g $cm^{-3}$ for soil, and 0.7 g $cm^{-3}$ for compost. The file also includes the average percentage variation in crop productivity, as well as the SOM (%) values reported for both the control (CTRL, no fertilization) and the MSWC treatments. These values were extracted directly from the text or tables of the original publications; no data were estimated from figures. Only comparisons between MSWC alone and a CTRL treatment with no added amendments were considered. Where available, the dataset also reports the country of the experiment; the soil texture classified according to the USDA system; the chemical composition of MSWC (pH, EC, total C, total N, total P, total K), from which C/N was derived; and the analytical results on MSWC heavy metals, pathogens, and microplastics.

Table S10: Descriptive statistics of MSWC composition across the 115 reviewed studies. Values are reported on a dry-matter basis (Total C, Total N, Total P, Total K). EC is electrical conductivity. SE = standard error of the mean; IQR = interquartile range (Q3 - Q1). pH and C/N are dimensionless. Data were computed using available observations only; sample size may vary by variable.

| Variable | Articles with data (n) | Unit | Mean | SE | Min | Q1 | Median | Q3 | Max | IQR |
|---|---|---|---|---|---|---|---|---|---|---|
| pH | 74 | - | 7.83 | 0.08 | 6.32 | 7.40 | 7.90 | 8.30 | 9.50 | 0.90 |
| EC | 49 | dS $m^{-1}$ | 6.59 | 0.73 | 0.40 | 2.88 | 5.10 | 9.50 | 23.20 | 6.62 |
| Total C | 77 | % (d.m.) | 23.34 | 1.25 | 8.70 | 15.40 | 21.50 | 28.50 | 89.25 | 13.10 |
| Total N | 90 | % (d.m.) | 1.71 | 0.08 | 0.51 | 1.25 | 1.67 | 2.15 | 4.97 | 0.90 |
| C/N | 78 | - | 14.70 | 0.80 | 6.70 | 10.20 | 12.00 | 16.20 | 46.70 | 6.00 |
| Total P | 60 | % (d.m.) | 0.53 | 0.05 | 1.00 | 0.21 | 0.71 | 1.71 | 1.71 | 0.50 |
| Total K | 58 | % (d.m.) | 0.89 | 0.10 | 0.10 | 0.40 | 0.72 | 1.09 | 3.79 | 0.69 |

Table S11: Nutrient loads supplied by MSWC across reviewed field studies (kg $ha^{-1}$ $yr^{-1}$). Statistics were computed from available observations only (mean ± SE; IQR = Q3 − Q1). Loads were derived from reported MSWC composition (Total C, N, P, K on a dry-matter basis, elemental P and K) and the average application rate (t $ha^{-1}$ $yr^{-1}$); values represent total applied nutrient, not plant-available fractions. Variation reflects both dose and material composition.

| Variable | Unit | Mean | SE | Min | Q1 | Median | Q3 | Max | IQR |
|---|---|---|---|---|---|---|---|---|---|
| Total C | kg $ha^{-1}$ $yr^{-1}$ | 6760.2 | 760.0 | 383.6 | 2930.5 | 5467.0 | 9693.6 | 31959 | 6763.1 |
| Total N | kg $ha^{-1}$ $yr^{-1}$ | 485.1 | 43.7 | 41.2 | 198.0 | 416.0 | 723.9 | 1416 | 525.9 |
| Total P | kg $ha^{-1}$ $yr^{-1}$ | 140.4 | 21.8 | 0.1 | 42.8 | 106.5 | 162.4 | 682 | 119.7 |
| Total K | kg $ha^{-1}$ $yr^{-1}$ | 218.3 | 33.3 | 6.4 | 67.3 | 149.3 | 298.2 | 960 | 230.9 |

Table S12: Distribution of soil texture classes (USDA) across the experimental sites. Values report the percentage of studies in each class; "Data not available" indicates articles that did not report soil texture.

| Soil texture (USDA) | % |
|---|---|
| Sandy loam | 19.6 |
| Loam | 11.7 |
| Clay | 8.6 |
| Sand | 8.6 |
| Silt loam | 8.6 |
| Clay loam | 7.8 |
| Silty clay | 5.5 |
| Loamy sand | 4.7 |
| Sandy clay loam | 2.3 |
| Silty clay loam | 2.3 |
| Data not available | 20.3 |

Table S13: Data coverage by contaminant category across the 115 reviewed studies. Values indicate the number of articles reporting at least one measurement and the corresponding share of the total.

| Contaminant | Articles reporting data (n) | Share of total (%) |
|---|---|---|
| Heavy metals | 66 | 57.4 |
| Pathogens | 1 | 0.9 |
| Microplastics | 1 | 0.9 |

Table S14: Heavy-metal concentrations in MSWC on a dry-matter basis. Values are reported as descriptive statistics across the reviewed studies (Mean $\pm$ SE; Min, Q1, Median, Q3, Max; IQR = Q3 − Q1). Units are mg $kg^{-1}$ (d.m.). Figures were computed using available observations only; sample size may differ by element.

| Variable | Unit | Mean | SE | Min | Q1 | Median | Q3 | Max | IQR |
|---|---|---|---|---|---|---|---|---|---|
| As | mg $kg^{-1}$ (d.m.) | 6.62 | 2.43 | 0.950 | 1.70 | 5.95 | 10.20 | 15.0 | 8.50 |
| Cd | mg $kg^{-1}$ (d.m.) | 3.50 | 0.90 | 0.001 | 0.80 | 1.40 | 3.37 | 26.0 | 2.57 |
| Cr | mg $kg^{-1}$ (d.m.) | 49.98 | 12.06 | 0.001 | 1.88 | 20.70 | 67.94 | 150.0 | 66.06 |
| Cu | mg $kg^{-1}$ (d.m.) | 169.95 | 15.65 | 0.040 | 90.54 | 128.55 | 243.30 | 403.0 | 152.76 |
| Hg | mg $kg^{-1}$ (d.m.) | 1.20 | 0.10 | 1.100 | 1.10 | 1.20 | 1.25 | 1.4 | 0.15 |
| Ni | mg $kg^{-1}$ (d.m.) | 66.94 | 12.38 | 4.800 | 11.00 | 33.05 | 94.00 | 217.0 | 83.00 |
| Pb | mg $kg^{-1}$ (d.m.) | 219.63 | 30.07 | 7.050 | 52.65 | 159.40 | 267.50 | 670.0 | 214.85 |
| Zn | mg $kg^{-1}$ (d.m.) | 407.64 | 39.29 | 0.490 | 201.50 | 307.10 | 566.49 | 1372.0 | 364.99 |

Table S15: Compliance of MSWC heavy-metal concentrations with regulatory thresholds. Limits refer to the EU Fertilising Products Regulation (EU) 2019/1009 for organic soil improvers (mg $kg^{-1}$ on a dry-matter basis). Percentages are calculated only over articles reporting at least one measurement for the given metal (n = number of such articles; total articles reviewed = 115).

| Metal | Limit (mg $kg^{-1}$ d.m.) | Articles with data (n) | Share > limit (%) | Share ≤ limit (%) |
|---|---|---|---|---|
| As | 40 | 6 | 0.0 | 100.0 |
| Cd | 2 | 41 | 43.9 | 56.1 |
| Cr | 2 | 31 | 74.2 | 25.8 |
| Cu | 300 | 54 | 22.2 | 77.8 |
| Hg | 1 | 3 | 100.0 | 0.0 |
| Ni | 50 | 38 | 31.6 | 68.4 |
| Pb | 120 | 51 | 52.9 | 47.1 |
| Zn | 800 | 57 | 7.0 | 93.0 |

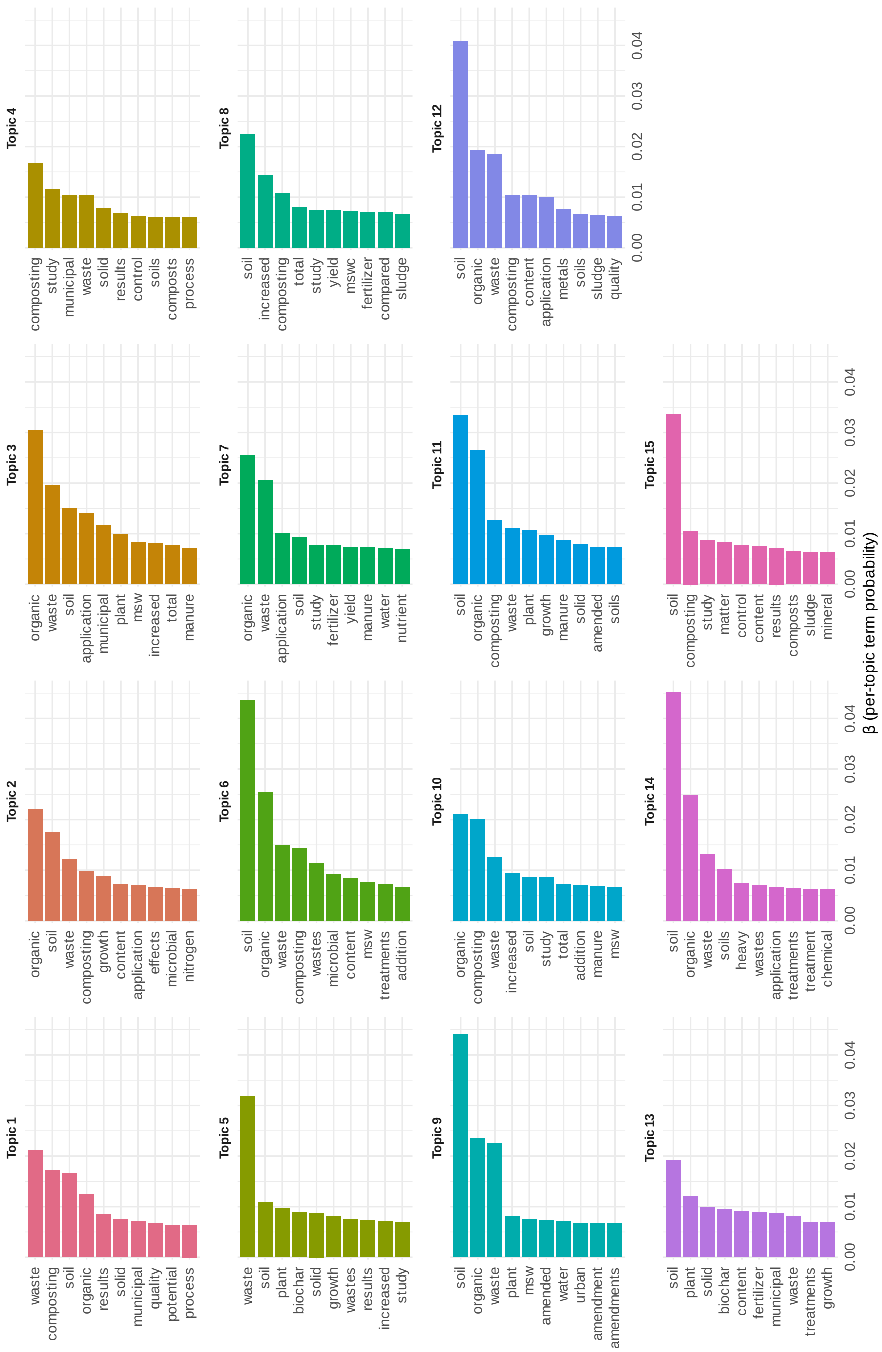


Figure S4: The results of topic modeling as obtained by Latent Dirichlet Allocation on the abstracts of the reviewed articles. Each panel shows topic x, sensu LDA modeling . For each panel, the terms listed on y axis indicate the words most associated with that specific topic, while beta on x axis indicates the probability of each term to belong to the topic.

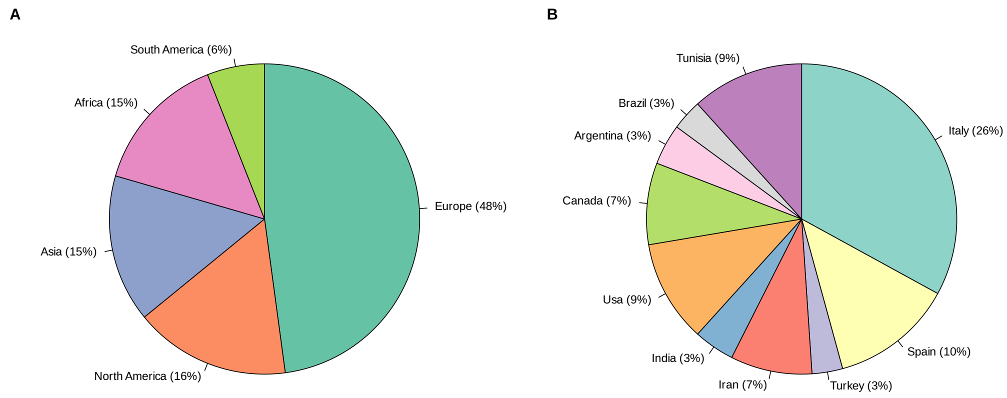


Figure S5: Geographic distribution of the reviewed MSWC studies (n = 115). (A) Share of studies by continent. (B) Top 10 countries by share of studies. Percentages are computed over all reviewed articles; multi-country studies contribute to each listed country, so panel totals sum to 100%. Abbreviation: MSWC = municipal solid-waste compost.

# 5 Circular Soil Amendment Strategies in Mediterranean Organic Systems: A two-year field study in the Montepaldi Long-Term Experiment, Tuscany

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## 5.1 Abstract

Returning locally available organic materials to soil is a cornerstone of circular, climate-smart agriculture in Mediterranean systems, yet evidence on the agronomic value of small, repeated inputs under stockless organic management remains limited. We conducted a two-year field trial within the Montepaldi Long-Term Experiment (Tuscany, Italy) to test whether low-dose amendments (2 t $ha^{-1}$ per application, calibrated to the on-farm supply of vine prunings) improve soil functions and cereal performance. Four amendments were evaluated: three pruning-based composts produced from vine prunings co-composted with alfalfa (PRUN, without inoculants; COMM, inoculated with a commercial accelerator; INDG, inoculated with Indigenous Microorganisms) and a vermicompost from spent coffee grounds (VERM), all compared to an unamended CTRL. Indicators included available P, aggregate stability, penetration resistance, earthworm metrics, crop development, grain yield, and yield components. Available P declined over time across all treatments, with no amendment effect, highlighting the need for targeted P replenishment in low-input organics. Amendments increased early-time resistance to slaking relative to the control, while 10-min stability and penetration resistance were unchanged, indicating a surface-focused, short-term structural benefit at low rates. Biological responses were amendment-quality dependent: VERM increased earthworm mean body mass versus control; pruning-based composts did not. Crop physiology and phenology were unaffected. By contrast, grain yield increased in the second year for all amendments, with gains driven by spike density rather than unit yield components, consistent with a cumulative structural contribution rather than improved grain filling. Small but sustained applications of residue-derived amendments can deliver incremental, context-dependent benefits to soil function and yield in established organic systems, provided they are maintained across seasons and complemented by explicit P management.

## 5.2 Keywords

Agroecology, Mediterranean agriculture, Circularity, Agricultural residues, Soil fertility, Crop productivity

## 5.3 Introduction

Across the Mediterranean, decades of agricultural intensification and the decoupling of crops from livestock have curtailed the return of organic materials to arable soils (Feller et al. 2012; Manlay et al. 2007). The disruption of these on-farm nutrient loops has contributed to a steady decline in soil organic matter (SOM) - a keystone of soil fertility, aggregation, water regulation, and biological functioning (Lal 2004) - with documented consequences for erosion risk, drought sensitivity, and long-term productivity (Ferreira et al. 2022; Montanarella et al. 2016). At the same time, modern farming generates large volumes of plant-based residues that are often underutilized or disposed of through burning or unmanaged, adding to atmospheric pollution and greenhouse gas emissions while losing a potentially valuable resource (Lou and Nair 2009; Medina et al. 2015). Beyond crop residues, urban and agro-industrial organic wastes also represent sizeable, locally available biomass streams. Re-closing the carbon and nutrient cycles by returning suitably treated residues and organic waste to fields is therefore central to both soil restoration and climate-smart management in Mediterranean agriculture (Cao et al. 2023; Kebede et al. 2023; O'Connor et al. 2024).

Agroecology and the circular economy offer a coherent framework to guide this transition, emphasizing the recycling of locally available biomass, reduced dependence on external inputs, and the valorization of on-farm resources (Altieri and Nicholls 2017; FAO 2018). Within this framework, composting and vermicomposting stand out as practical routes to transform heterogeneous organic wastes into agronomically useful amendments. Compost applications are known to promote soil aggregation, increase cation exchange capacity, and build organic carbon stocks (Diacono and Montemurro 2010). They can also enhance soil water-holding capacity—an increasingly critical function under climate scenarios characterized by prolonged dry spells and intense rainfall events (Bernal et al. 2009; Iglesias and Garrote 2015; Smith et al. 2020). Vermicompost, produced through earthworm processing of organic substrates, typically yields a finer, microbially active material with high moisture retention and low bulk density that can improve porosity and, in some systems, stimulate plant growth at relatively modest doses (Dominguez and Edwards 2010; Lazcano and Dominguez 2011; Lim et al. 2012). At the same time, the agronomic response to organic amendments is markedly feedstock- and process-dependent: lignin-rich residues such as vine prunings may release nutrients slowly and show limited short-term effects, whereas low C/N, more labile inputs can elicit stronger biological responses (Bernal et al. 2009; Diacono and Montemurro 2010).

Mediterranean arable systems add two layers of complexity. First, water availability often constrains growth more than nutrients, with crop performance tightly linked to near-surface structure and moisture dynamics (Poorter et al. 2012; Rouphael et al. 2012). Second, many organic systems are stockless, lacking farmyard manure and relying instead on crop residues and external inputs. In these contexts, aligning amendment rates with locally generated biomass becomes a strategic choice: it respects circularity principles, contains costs, and tests whether small, repeated inputs can accumulate benefits over time without creating fertilization pulses or salinity issues. Viticultural landscapes are emblematic: vineyard prunings represent several tonnes per hectare annually and are widely available on-farm, yet they are frequently burned (Florindo et al. 2022). Their conversion into compost - either alone or with targeted inocula - offers a pathway to return carbon to soil while avoiding imports of external organic matter (Lucchetta et al. 2025).

Long-term experiments provide a critical setting to assess such strategies under realistic management. The Montepaldi Long-Term Experiment (MoLTE) in Tuscany offers a mosaic of stockless arable systems with decades of consistent management, enabling the evaluation of residue-derived amendments on both soil functions and crop performance under Mediterranean conditions and supporting integrative assessment frameworks developed for these systems (Pacini et al. 2023). Notably, previous evidence at MoLTE and similar sites indicates that Available P can decline over time under low-input organic management unless specifically replenished (Diacono and Montemurro 2010; Pantani et al. 2022), underscoring the need to disentangle structural/biological benefits from direct nutrient supply when testing low rates.

Building on these premises, we conducted a two-year field trial in the organic systems of MoLTE to evaluate the effects of repeated low-dose applications (2 t ha$^{-1}$ per application) of four residue-derived amendments on soil fertility and cereal productivity. The amendments comprised three composts produced from vine prunings (with or without inoculants during composting process, such as a commercial composting booster and Indigenous Microorganisms, $IMO_2$) and a vermicompost from spent coffee grounds; an unamended control was included. The 2 t ha$^{-1}$ rate was calibrated to the average, locally available biomass of vine prunings per hectare in order to (i) adhere to circular-economy principles and on-farm feasibility, (ii) avoid reliance on imported organic matter, and (iii) probe whether small, repeated inputs can deliver cumulative structural and biological benefits under stockless organic management.

We specifically asked whether these low-dose, residue-derived amendments would: (i) modify key soil fertility indicators - chemical (available P), physical (aggregate stability, penetration resistance), and biological (earthworm metrics); and (ii) translate into changes in crop development and yield components across two consecutive winter cereal cycles.

Given the feedstock differences, we expected contrasting biological responses, limited short-term changes in available P at such low rates, and the possibility of lagged agronomic responses consistent with cumulative improvements in near-surface structure rather than with direct effects on grain filling.

By grounding amendment rates in locally produced residues and testing them under field conditions in established organic systems, this study aims to inform circular, site-specific strategies for restoring soil function and sustaining crop performance in Mediterranean arable agriculture.

## 5.4 Material and Methods

### 5.4.1 Description of the Experimental Site

The study was conducted at the Montepaldi Long-Term Experiment (MoLTE; San Casciano in Val di Pesa, Florence, Italy; 43°40 16 N, 11°09 08 E; 90 m a.s.l.), a Mediterranean site established in 1991 and continuously monitored since then. The platform covers ~15 ha on a gently sloping surface and is subdivided into ten ~1.3 ha plots. The main soil physico-chemical characteristics of the Montepaldi Long Term Experiment are shown in Table S16.

MoLTE hosts three stockless arable systems (organic "OldOrg", formerly integrated "NewOrg" converted to organic in 2001, and a conventional high-input system). Because our work targeted organic management, we operated exclusively within the OldOrg and NewOrg systems.

Our experiment included two consecutive winter cereal cycles on these organic fields: spelt (*Triticum spelta*) in 2023/24 and common wheat (*Triticum aestivum*) in 2024/25. Table S17 shows additional agronomic details.

### 5.4.2 Description of the experimental set-up

A randomized complete block design was implemented. The experiment comprised four organic amendments (PRUN, COMM, INDG and VERM) and an unamended control (CTRL). The first three amendments (PRUN, COMM and INDG) were produced from vine prunings + alfalfa (1:1 w/w) composted on site. Specifically, PRUN consisted of composted vine prunings without additives; COMM treatment included vine prunings composted with 600 g $m^{-3}$ of a commercial composting accelerator; and INDG treatment involved composting vine prunings inoculated with Indigenous Microorganisms ($IMO_2$), following Korean Natural Farming techniques. The $IMO_2$ culture was prepared through two stages ($IMO_1$, collection, and $IMO_2$, propagation), and applied to the compost pile at a rate of 500 g diluted in 10 L of water. All compost piles were equipped with three internal data loggers and were routinely turned and moistened to maintain aerobic conditions and stimulate microbial activity. The fourth amendment, VERM, was a vermicompost from spent coffee grounds (Funghi Espresso Società Agricola s.r.l.), differing in origin and composition from the pruning-based composts. Main physico-chemical properties of the four amendments are reported in Table 2.

The four amendmends were applied in the fields twice, in October 2023 and October 2024 (Table S17) at the dose of 2 t $ha^{-1}$ for each application. The 2 t $ha^{-1}$ rate was set to mirror the average on-farm availability of vine prunings per hectare, in keeping with circular-economy

Table 2: Averages (± standard deviation) of the physico-chemical qualities of the organic amendments, determined according to internationally recognized standard procedures (ISO, TMECC) (n = 3).

| Treatment | Density (kg $L^{-1}$) | Moisture (%) | pH | EC (mS $cm^{-1}$) | C (%) | N (%) | C/N |
|---|---|---|---|---|---|---|---|
| PRUN | 0.13 (0.01) | 13.2 (0.25) | 6.9 (0.05) | 0.90 (0.36) | 43.9 (0.67) | 0.80 (0.03) | 57.4 (3.39) |
| COMM | 0.13 (0.01) | 13.5 (0.40) | 6.9 (0.02) | 0.80 (0.02) | 44.5 (0.24) | 0.80 (0.02) | 58.0 (1.61) |
| INDG | 0.15 (0.01) | 12.8 (0.06) | 7.0 (0.01) | 0.70 (0.02) | 43.6 (1.34) | 0.70 (0.05) | 62.6 (3.03) |
| VERM | 0.49 (0.01) | 67.9 (0.78) | 5.8 (0.05) | 0.90 (0.06) | 42.8 (1.53) | 5.00 (0.19) | 8.5 (0.03) |

principles and farm feasibility; this avoids importing external organic matter and allows testing cumulative structural/biological effects of small, repeated inputs. One field per each organic system – 0.5445 ha per field – was divided in 3 horizontal strips (REP, Fig. S6). In each strip, 5 plots were randomly assigned to one level of treatments (TRT). Within each plot, 20 polygons with equivalent area were drawn (Walvoort et al. 2025) and a couple of xy coordinates were randomly generated within each polygon (Fig. S7). Finally, 4 xy coordinates were randomly selected among the 20 couples (SUB-REP) resulting up to 120 sampling point (2 fields $*$ 3 REP $*$ 5 TRT $*$ 4 SUB-REP) at which indicators were repeatedly sampled over TIME (Table S18).

### 5.4.3 Soil fertility indicators

**5.4.3.1 Chemical fertility** The soil was sampled in October 2023 and 2024 and in June 2025. For each sample available P was measured (Olsen 1954), and, after carbonate removal, soil organic C (SOC) and total N were measured by flash combustion with an elemental analyzer.

**5.4.3.2 Physical fertility** Wet-aggregate stability was assessed with the SLAKES smartphone application, which quantifies (i) the initial slaking response immediately after rapid wetting and (ii) the subsequent wet stability after a fixed immersion period (600 sec). The app extracts projected aggregate areas from standardized images and reports stability indices based on area ratios through time (i.e., larger area loss indicates greater disintegration). This image-based approach and its performance relative to conventional laboratory methods are described elsewhere and have been formally evaluated for agronomic soils (Fajardo et al. 2016; Flynn et al. 2020).

For each sampling location, intact surface aggregates were collected at two time points: T0 (October 2023, before amendment application) and T2 (June 2025, after two amendment applications). Following app guidelines, aggregates were gently placed on a Petri dish with 50 mL of distilled water; images were captured at the moment of immersion (early slaking) and after 10 min (overall wet stability). From the app outputs, we retained the corresponding stability indices (area-ratio–based scores) for each time point.

To isolate treatment effects on structural change over the experiment, the primary response variable was the change in stability ($\Delta$) computed at each location as T2 minus T0 for both the early-slaking index and the 10-min stability index. These $\Delta$ values were then used in the statistical analyses described below.

Regarding soil penetration resistance (0-50 cm) was performed with an hand penetrometer (Eijkelkamp, Netherlands). During the sampling session, four samples were collected for each SUB-REP, resulting in a total of 480 measurements.

**5.4.3.3 Biological fertility** Earthworm abundance was assessed using the VESS method (Ball et al. 2007), extracting a soil cubic block with 30 cm side and carefully breaking it in the field. During examination, all individuals were counted and assigned to age classes (baby, young, adult). In addition, the number of visible burrow openings on the block faces was recorded as a proxy for earthworm activity, given that *Hormogaster samnitica* - the predominant species at the site - occurs at very low densities and is therefore difficult to detect directly. After enumeration, each captured earthworm was gently blotted to remove surface moisture and weighed (fresh mass) using a portable balance.

### 5.4.4 Productivity indicators

**5.4.4.1 Crop development and physiology** To measure crop development and physiology indicators, on plant near each SUB-REP was selected and followed through the season. Tillers number was counted once for each plant at the end of the tillering phase. From this assessment crop phenology, using the BBCH scale (Hess et al. 1997), and plant height (cm) was recorded routinely until physiological maturity. In addition, leaf optical traits were measured once during the season on the same plants using a Dualex leaf-clip meter (METOS Dualex; Pessl Instruments, Austria), providing chlorophyll index (Chl), flavonol index (Flav) and Nitrogen Balance Index (NBI).

**5.4.4.2 Yield** Grain yield and yield components were determined using a square metal frame (50 × 50 cm; 0.25 $m^2$) placed adjacent to the pre-randomized sampling point. For each SUB-REP and both crops (*Triticum dicoccum* and *Triticum aestivum*), all plants within the frame were cut at the base and transported to the laboratory.

In the lab, spikes were counted, then samples were threshed; grain was weighed (dry mass) after oven-drying at 60°C to constant weight, and seeds were counted. From these measurements we derived:

- Grain yield (t $ha^{-1}$) by scaling dry grain mass from 0.25 $m^2$ to 1 ha;
- Spike density (spikes $m^{-2}$) from spike counts per 0.25 $m^2$;
- Seeds per spike, as total seed number divided by spike count;
- Mean spike mass (g $spike^{-1}$), as dry grain mass divided by spike count;
- 100-seed weight (g) from counted subsamples.

All yield metrics are reported on a dry-matter basis.

### 5.4.5 Statistical Analysis

All statistical analyses were performed with R version 4.5.0 (R Core Team 2023). Data handling used dplyr and the tidyverse packages (Wickham 2016), while figures were produced with lattice (Sarkar 2001).

Treatment effects were assessed with linear models (LMs) for continuous responses and generalized linear models (GLMs) for counts, with negative binomial or Gamma families adopted when appropriate. LM assumptions were checked by visual inspection of residual diagnostics and formally with Shapiro-Wilk (normality) and Breusch-Pagan (homoscedasticity) tests; when variance heterogeneity was detected, we fitted weighted least squares. For GLMs, dispersion was evaluated via simulated residuals with DHARMa package (Hartig et al. 2024) to detect over/under-dispersion. For each response, we followed a marginal-model hierarchy to identify the most parsimonious specification: first TRT $*$ YEAR, then TRT + YEAR, and finally TRT only.

Statistical significance was set at $p < 0.05$.

## 5.5 Results

### 5.5.1 Soil fertility indicators

**5.5.1.1 Chemical fertility** Across the 20-month experiment, the organic amendments did not measurably affect available P, nor did they modify its temporal trend. By contrast, available P declined significantly over time at -0.09 ppm month$^{-1}$ ($p = 0.016$, Fig. 12, resulting in almost -1.8 ppm across the study period. For soil organic C, only INDG increased ΔSOC (%) between T2 (June 2025) and T0 (October 2023) (+0.090, $p = 0.02$; Fig. 13).

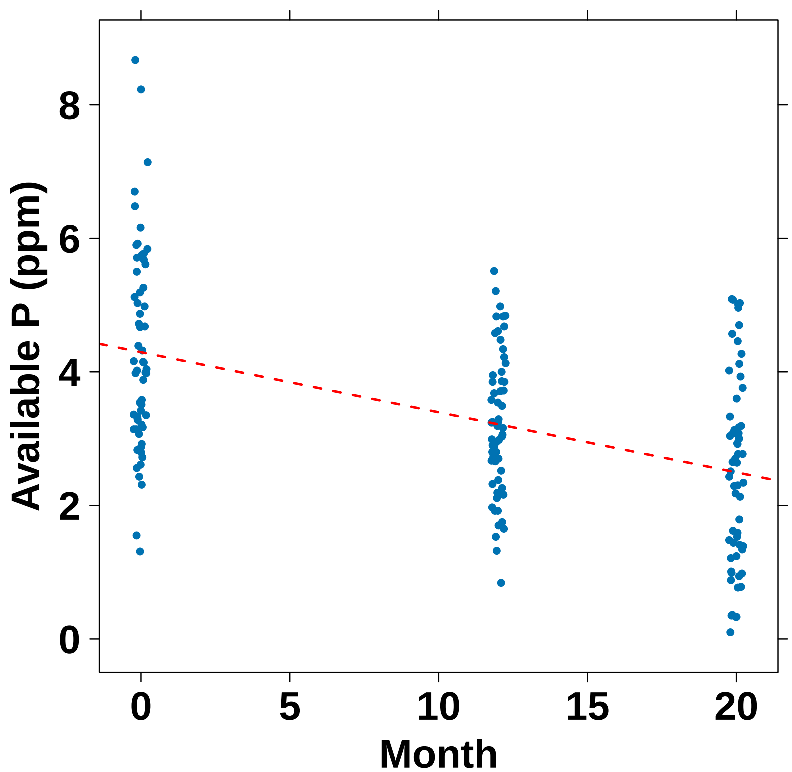


Figure 12: Temporal dynamics of soil available phosphorus (P, ppm) over the 20-month study period (October 2023 - June 2025). Individual points represent measurements collected at fixed sampling sites at each sampling date (0, 12, and 20 months). The dashed line indicates the overall linear trend estimated across all observations, highlighting a significant decline in available P over time.

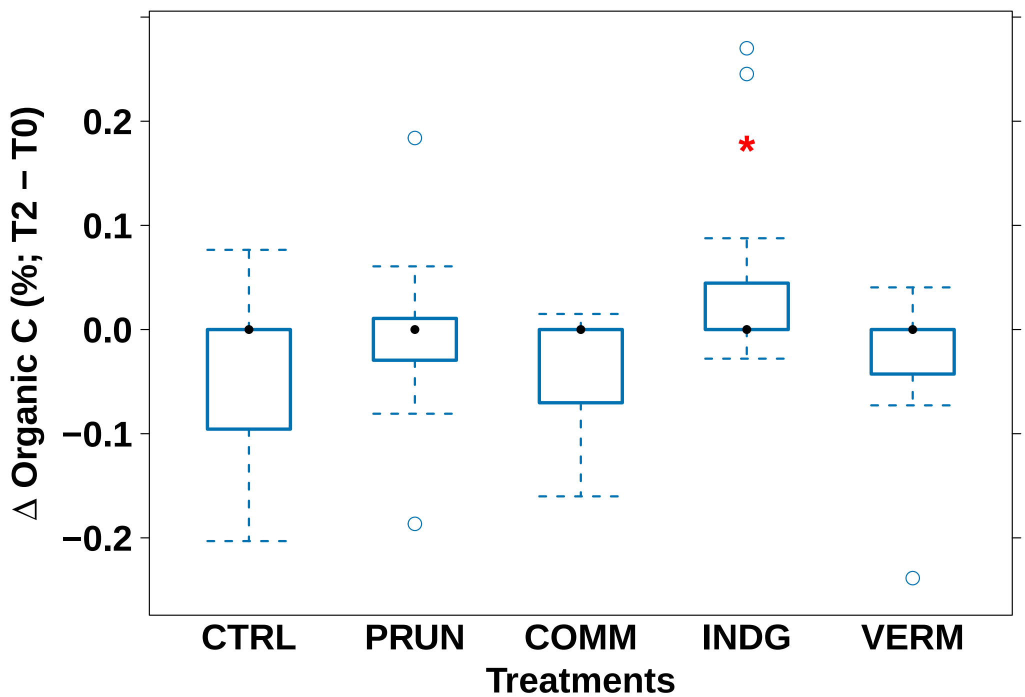


Figure 13: Change (Δ) in soil organic carbon content (%) between T2 (June 2025) and T0 (October 2023) across treatments. Asterisks indicate statistical significance of pairwise comparisons against CTRL: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

**5.5.1.2 Physical fertility** Using the SLAKES protocol, which quantifies early-time slaking (aggregate breakdown upon rapid wetting) and a later overall wet aggregate stability after a fixed immersion period, we detected marked treatment effects at the beginning of wetting. Immediately after immersion, all amended soils showed greater resistance to rapid wetting than the unamended control (Fig. 14), indicating an improvement in the short-term stability of surface aggregates. By contrast, no treatment effect was evident on the overall wet aggregate stability after 10 min of immersion (Fig. S8).

Soil penetration resistance was comparable among treatments (Fig. S9).

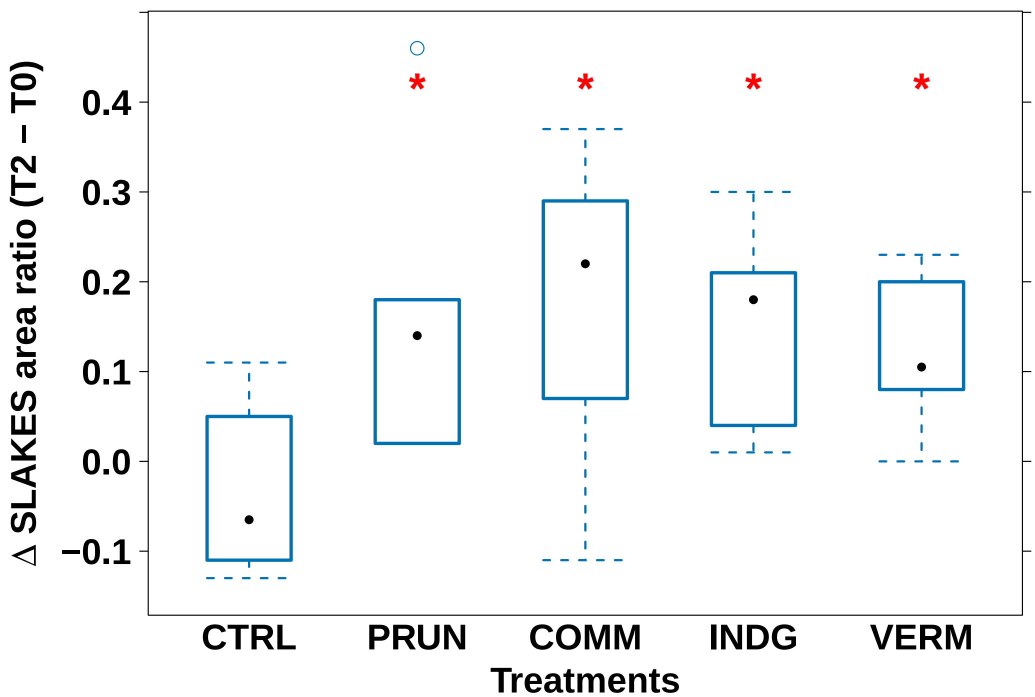


Figure 14: Change (Δ) in early-time aggregate stability upon rapid wetting (SLAKES area-ratio index; T2 − T0) across treatments. Asterisks indicate statistical significance of pairwise comparisons against CTRL: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

**5.5.1.3 Biological fertility** Earthworm mean body mass of the individuals found differed among amendments: individuals recovered from VERM plots were, on average, heavier than those from CTRL (+0.8 g), whereas PRUN, COMM and INDG did not differ from CTRL (Fig. 15).

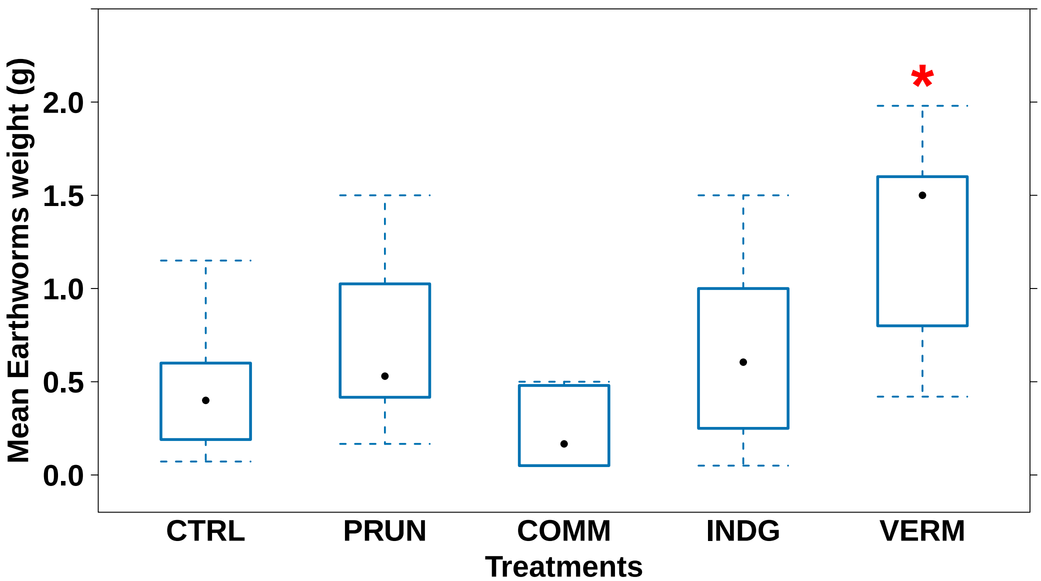


Figure 15: Mean earthworms weight (g) collected in field plots by treatments. Asterisks indicate statistical significance of pairwise comparisons against CTRL: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

Used as a proxy of earthworms activity, the number of biopores did not differ significantly among treatments (Fig. S10). Finally, earthworm occurrence was primarily driven by year, with a higher detection probability in 2025 than in 2024, and no consistent amendment effect has been detected (Fig. S11).

### 5.5.2 Productivity

**5.5.2.1 Crop development and physiology** Across both cropping cycles, phenological and physiological indicators did not differ among treatments. In particular, BBCH growth stages, plant height, Dualex-derived leaf traits (chlorophyll content, flavonol index, nitrogen balance index), and tiller number showed no consistent treatment effects (Figs. S12 - S15).

**5.5.2.2 Grain yield and yield components** Grain yield increased under organic amendments in the second cropping cycle, whereas no treatment effects were detected in the first (Fig. 16. The two cropping cycles involved different cereal species, which limits direct comparison between years. In 2025, all treatments had significantly higher yield than the control (p <

0.05).

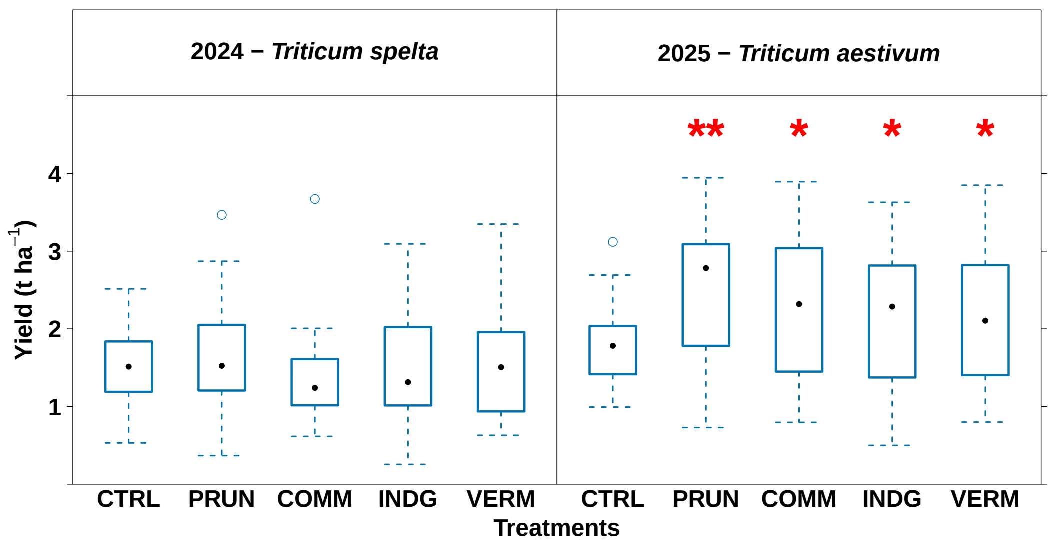


Figure 16: Grain yield (t $ha^{-1}$) by treatment for the two cropping cycles: 2024 - Triticum spelta and 2025 - Triticum aestivum.ht (g) collected in field plots by treatments. Asterisks indicate statistical significance of pairwise comparisons against CTRL: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

This pattern was partly mirrored by spike density: PRUN and COMM had more spikes $m^{-2}$ than the CTRL, whereas INDG and VERM not showed significant differences (Fig. 17). By contrast, unit-yield components such as 100-seed weight, seeds per ear and average ear mass
2435 were largely stable across treatments and years (Fig. S16).

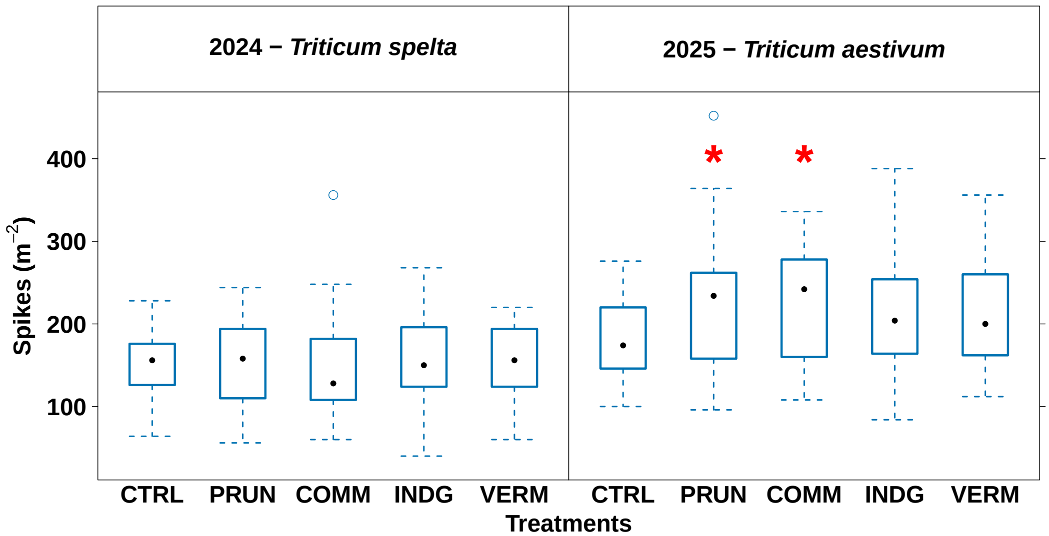


Figure 17: Spikes number ($m^{-2}$) by treatment for the two cropping cycles: 2024 - Triticum spelta and 2025 - Triticum aestivum.Asterisks indicate statistical significance of pairwise comparisons against CTRL: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

## 5.6 Discussion

### 5.6.1 Effects of organic amendments on soil fertility

The first objective of this study was to assess whether repeated low-dose applications of different organic amendments could improve soil fertility indicators in long-term organically managed fields. Our findings indicate that chemical fertility, as measured by available P, was unaffected by treatments, while a significant temporal decline was observed across all plots. This result suggests that, under Mediterranean organic systems with limited external inputs, P availability tends to decline over time unless replenished, as previously reported for stockless rotations at the same site (Pantani et al. 2022) or in similar environments (Diacono and Montemurro 2010). The absence of amendment effects can be explained by the very low application rate (2 t $ha^{-1}$ $year^{-1}$), insufficient to alter the soil P pool in the short term. Previous studies have highlighted that detectable increases in available P generally occur at much higher application rates or after multiple years of amendment inputs (Martínez-Blanco et al. 2013). By contrast, soil organic carbon showed a selective treatment response: only the INDG treatment produced a measurable increase in ΔSOC between October 2023 and June 2025, while other amendments were indistinguishable from the CTRL. The INDG compost, obtained from vine prunings inoculated with Indigenous Microorganisms ($IMO_2$) following Korean Natural Farming techniques, likely promoted greater microbial activity and organic matter transformation during decomposition, leading to enhanced C stabilization in soil. Similar positive effects of microbially enriched composts have been observed in Mediterranean soils with low baseline organic matter (Diacono and Montemurro 2010), suggesting that microbial inoculation can increase the functional efficiency of lignin-rich pruning composts even at low application rates.

Regarding physical fertility, we observed a clear positive effect of amendments on early-time aggregate stability (slaking resistance), whereas the longer-term wet stability after 10 minutes was not significantly influenced. This pattern suggests that organic inputs conferred a short-term protective effect on surface aggregates against rapid wetting, possibly through enhanced organic coatings or transient microbial activity (Six et al. 2004). However, the lack of effect on the overall stability index points to limited structural reorganization of aggregates, which is consistent with both the low input rate and the relatively short duration of the trial. The absence of differences in penetration resistance among treatments further supports the conclusion that, under these conditions, soil mechanical properties remain largely unaltered.

Biological fertility indicators provided more nuanced insights. Earthworm biomass was higher in VERM plots than in the control, while pruning-based composts did not differ. This selective response likely reflects the contrasting composition of amendments: vermicompost from coffee grounds supplied higher N and more labile organic matter (low C/N, high moisture), which are known to stimulate earthworm feeding and growth (Curry and Schmidt 2007; Ducasse et al. 2025). By contrast, lignin-rich pruning based compost (PRUN, COMM, INDG) provided limited short-term benefits for soil fauna, confirming that amendment quality is crucial in shaping biological responses. Earthworm occurrence itself was more strongly influenced by inter-annual variability than by treatments, which is consistent with the ecology of *Hormogaster samnitica* and the inherent challenges in detecting its populations at low densities (Capowiez et al. 2024; Omodeo and Rota 2008).

### 5.6.2 Effects on crop development and productivity

The second objective was to test whether organic amendments could enhance crop growth and yield components. No treatment effects were observed on crop phenology, plant height, or leaf optical traits across both cropping cycles. This outcome reflects the limited contribution of low-dose organic inputs to aboveground physiological traits in cereals, especially within long-term organic systems where soil fertility is already stabilized (Seufert et al. 2012). Similar findings have been reported in other Mediterranean trials, where moderate organic inputs did not significantly alter vegetative growth parameters (Morra et al. 2021).

In contrast, grain yield exhibited a significant response during the second year, when all amendments outperformed the control. This delayed effect suggests a cumulative contribution of amendments, possibly through cumulative enhancements in near-surface aggregate stability against rapid wetting. The fact that yield differences were mainly driven by spike density, rather than unit yield components (seed weight, seeds per spike, spike mass), points to an influence on tiller survival or spike initiation rather than on grain filling. Such a mechanism is coherent with literature showing that organic inputs can enhance yield stability by modulating plant density and stand uniformity, even at relatively low rates (Tognetti et al. 2005; Zhang et al. 2024). Importantly, this finding highlights that even minimal amendment inputs may become agronomically relevant when sustained across consecutive years.

### 5.6.3 Limitations and future perspectives

Despite these encouraging results, several limitations must be acknowledged. The amendments were applied at very low doses compared to common agricultural practice, which explains the limited and treatment-specific effects observed. Moreover, the study covered only two cropping cycles; longer monitoring would be necessary to evaluate cumulative benefits and potential legacy effects on soil nutrient pools and structure. Our measurements also focused on selected fertility indicators (mainly P, organic C, aggregate stability, earthworm metrics), while other relevant parameters such as microbial activity, or potential contaminants (heavy metals, microplastics) were not assessed. Notably, the increase in soil organic carbon observed only under the INDG treatment suggests that microbial inoculation may enhance C stabilization even at low compost rates, a hypothesis that warrants targeted investigation through combined C fractionation and microbiological analyses. These aspects deserve future investigation, particularly given the variability in compost composition depending on feedstock and processing (Diacono and Montemurro 2010; Ducasse et al. 2025).

Overall, our findings demonstrate that repeated low-dose applications of organic amendments can contribute to soil biological activity and crop yield in long-term organic systems, although effects are modest and context-dependent. Future studies should evaluate higher application rates, a broader range of soil fertility indicators, and interactions with crop rotations under diverse Mediterranean conditions.

## 5.7 Conclusions

In long-term organic arable systems, repeated low-dose inputs (2 t $ha^{-1}$ $yr^{-1}$) deliver incremental benefits that emerge over time. Across two cropping cycles, available P declines irrespective of treatment, indicating that P must be actively replenished under Mediterranean conditions with limited external inputs. At the same time, the selective increase in soil organic carbon under the INDG treatment indicates that microbial inoculation of lignin-rich composts may promote organic matter stabilization even at modest application rates. Amendments improve early-time resistance to slaking, pointing to a short-term protection of surface aggregates, while overall wet stability (10 min) and penetration resistance remain unchanged, consistent with the low dose and short timeframe.

Soil biological responses are amendment-quality dependent. Vermicompost from coffee grounds increases earthworm mean body mass relative to the control, whereas pruning derived composts do not. This pattern highlights the role of organic matter lability and C/N characteristics in shaping soil faunal activity at low inputs.

Crop performance reflects these subtle soil changes with a time lag. Grain yield increases only in the second year and is driven by spike density, not by unit yield components. The evidence supports a cumulative structural/nutritional contribution of the amendments—consistent with enhanced near-surface aggregation against rapid wetting—rather than improved grain filling.

Overall, small but sustained applications can contribute to soil function and yield in established organic systems, provided they are maintained across seasons and coupled with targeted P management. Future work should extend the time horizon and explore higher rates, testing whether early structural gains translate into enduring aggregate stability and measurable changes in nutrient pools, while broadening the biological and chemical indicators to capture system-level responses.

## 5.8 References


Altieri MA, Nicholls CI (2017) Agroecology: A brief account of its origins and currents of thought in Latin America. Agroecology and Sustainable Food Systems 41:231–237. https://doi.org/10.1080/21683565.2017.1287147

Ball BC, Batey T, Munkholm LJ (2007) Field assessment of soil structural quality – a development of the Peerlkamp test. Soil Use and Management 23:329–337. https://doi.org/10.1111/j.1475-2743.2007.00102.x

Bernal MP, Alburquerque JA, Moral R (2009) Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresource Technology 100:5444–5453. https://doi.org/10.1016/j.biortech.2008.11.027

Cao X, Williams PN, Zhan Y, Coughlin SA, McGrath JW, Chin JP, Xu Y (2023) Municipal solid waste compost: Global trends and biogeochemical cycling. Soil & Environmental Health 1:100038. https://doi.org/10.1016/j.seh.2023.100038

Capowiez Y, Marchán D, Decaëns T, Hedde M, Bottinelli N (2024) Let earthworms be functional - Definition of new functional groups based on their bioturbation behavior. Soil Biology and Biochemistry 188:109209. https://doi.org/10.1016/j.soilbio.2023.109209

Curry JP, Schmidt O (2007) The feeding ecology of earthworms – A review. Pedobiologia 50:463–477. https://doi.org/10.1016/j.pedobi.2006.09.001

Diacono M, Montemurro F (2010) Long-term effects of organic amendments on soil fertility. A review. Agronomy for Sustainable Development 30:401–422. https://doi.org/10.1051/agro/2009040

Dominguez J, Edwards C (2010) Relationships between Composting and Vermicomposting. In: Edwards C, Arancon N, Sherman R (eds) Vermiculture Technology. CRC Press, pp 11–25

Ducasse V, Capowiez L, Peigne J, Capowiez Y (2025) Compost, digestate, and vermicompost from the recycling of urban biowaste have different impacts on earthworm behavior: A mesocosm study. Applied Soil Ecology 210:106095. https://doi.org/10.1016/j.apsoil.2025.106095

Fajardo M, McBratney AlexB, Field DJ, Minasny B (2016) Soil slaking assessment using image recognition. Soil and Tillage Research 163:119–129. https://doi.org/10.1016/j.still.2016.05.018

FAO (2018) The 10 Elements of Agroecology. Guiding the Transition to Sustainable Food and Agricultural Systems.

Feller C, Blanchart E, Bernoux M, Lal R, Manlay R (2012) Soil fertility concepts over the past two centuries: The importance attributed to soil organic matter in developed and developing countries. Archives of Agronomy and Soil Science 58:S3–S21. https://doi.org/10.1080/03650340.2012.693598

Ferreira CSS, Seifollahi-Aghmiuni S, Destouni G, Ghajarnia N, Kalantari Z (2022) Soil degradation in the European Mediterranean region: Processes, status and consequences. Science of The Total Environment 805:150106. https://doi.org/10.1016/j.scitotenv.2021.150106

Florindo T, Ferraz AI, Rodrigues AC, Nunes LJR (2022) Residual Biomass Recovery in the Wine Sector: Creation of Value Chains for Vine Pruning. Agriculture 12:670. https://doi.org/10.3390/agriculture12050670

Flynn KD, Bagnall DK, Morgan CLS (2020) Evaluation of SLAKES, a smartphone application for quantifying aggregate stability, in high-clay soils. Soil Science Society of America Journal 84:345–353. https://doi.org/10.1002/saj2.20012

Hartig F, Lohse L, leite M de S (2024) DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models

Hess M, Barralis G, Bleiholder H, Buhr L, Eggers Th, Hack H, Stauss R (1997) Use of the extended BBCH scale—general for the descriptions of the growth stages of mono; and dicotyledonous weed species. Weed Research 37:433–441. https://doi.org/10.1046/j.1365-3180.1997.d01-70.x

Iglesias A, Garrote L (2015) Adaptation strategies for agricultural water management under climate change in Europe. Agricultural Water Management 155:113–124. https://doi.org/10.1016/j.agwat.2015.03.014

Kebede T, Diriba D, Boki A (2023) The Effect of Organic Solid Waste Compost on Soil Properties, Growth, and Yield of Swiss Chard Crop (Beta vulgaris L.). The Scientific World Journal 2023:6175746. https://doi.org/10.1155/2023/6175746

Lal R (2004) Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. Science 304:1623–1627. https://doi.org/10.1126/science.1097396

Lazcano C, Dominguez J (2011) The use of vermicompost in sustainable agriculture: Impact on plant growth and soil fertility. In: Soil nutrients

Lim SL, Wu TY, Sim EYS, Lim PN, Clarke C (2012) Biotransformation of rice husk into organic fertilizer through vermicomposting. Ecological Engineering 41:60–64. https://doi.org/10.1016/j.ecoleng.2012.01.011

Lou XF, Nair J (2009) The impact of landfilling and composting on greenhouse gas emissions – A review. Bioresource Technology 100:3792–3798. https://doi.org/10.1016/j.biortech.2008.12.006

Lucchetta M, Zuluaga MYA, Monterisi S, Fattorini R, Gaiotti F, Cesco S, Pii Y (2025) Innovative approaches to soil health: Evaluating pruning waste compost, inoculated compost, and mineral fertilizer in a controlled growth chamber for sustainable vineyard practices. Frontiers in Sustainable Food Systems 9: https://doi.org/10.3389/fsufs.2025.1504761

Manlay RJ, Feller C, Swift MJ (2007) Historical evolution of soil organic matter concepts and their relationships with the fertility and sustainability of cropping systems. Agriculture, Ecosystems & Environment 119:217–233. https://doi.org/10.1016/j.agee.2006.07.011

Martínez-Blanco J, Lazcano C, Christensen TH, Muñoz P, Rieradevall J, Møller J, Antón A, Boldrin A (2013) Compost benefits for agriculture evaluated by life cycle assessment. A review. Agronomy for Sustainable Development 33:721–732. https://doi.org/10.1007/s13593-013-0148-7

Medina J, Monreal C, Barea JM, Arriagada C, Borie F, Cornejo P (2015) Crop residue stabilization and application to agricultural and degraded soils: A review. Waste Management 42:41–54. https://doi.org/10.1016/j.wasman.2015.04.002

Montanarella L, Pennock DJ, McKenzie N, et al (2016) World's soils are under threat. SOIL 2:79–82. https://doi.org/10.5194/soil-2-79-2016

Morra L, Bilotto M, Baldantoni D, Alfani A, Baiano S (2021) A seven-year experiment in a vegetable crops sequence: Effects of replacing mineral fertilizers with Biowaste compost on crop productivity, soil organic carbon and nitrates concentrations. Scientia Horticulturae 290:110534. https://doi.org/10.1016/j.scienta.2021.110534

O'Connor J, Mickan BS, Gurung SK, Bühlmann CH, Jenkins SN, Siddique KHM, Leopold M, Bolan NS (2024) Value of food waste-derived fertilisers on soil chemistry, microbial function and crop productivity. Applied Soil Ecology 198:105380. https://doi.org/10.1016/j.apsoil.2024.105380

Olsen SR (1954) Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. U.S. Department of Agriculture

Omodeo P, Rota E (2008) Earthworm Diversity and Land Evolution in Three Mediterranean Districts. Proceedings of the California Academy of Sciences Fourth Series Volume 59:65–83

Pacini GC, Bruschi P, Ferretti L, Santoni M, Serafini F, Gaifami T (2023) FunBies, a model for integrated assessment of functional biodiversity of weed communities in agro-ecosystem. Ecological Modelling 486:110529. https://doi.org/10.1016/j.ecolmodel.2023.110529

Pantani O-L, Ferretti L, Santoni M, Massenzio S, D'Acqui LP, Pacini GC (2022) Assessment of the impact of conventional and organic agroecosystems management options and conservation tillage on soil fertility at the Montepaldi Long Term Experiment, Tuscany. European Journal of Agronomy 140:126575. https://doi.org/10.1016/j.eja.2022.126575

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012) Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control.

New Phytologist 193:30–50. https://doi.org/10.1111/j.1469-8137.2011.03952.x

R Core Team (2023) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Rouphael Y, Cardarelli M, Schwarz D, Franken P, Colla G (2012) Effects of Drought on Nutrient Uptake and Assimilation in Vegetable Crops. In: Aroca R (ed) Plant Responses to Drought Stress. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 171–195

Sarkar D (2001) Lattice: Trellis Graphics for R

Seufert V, Ramankutty N, Foley JA (2012) Comparing the yields of organic and conventional agriculture. Nature 485:229–232. https://doi.org/10.1038/nature11069

Six J, Bossuyt H, Degryze S, Denef K (2004) A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage Research 79:7–31. https://doi.org/10.1016/j.still.2004.03.008

Smith P, Soussana J-F, Angers D, et al (2020) How to measure, report and verify soil carbon change to realize the potential of soil carbon sequestration for atmospheric greenhouse gas removal. Global Change Biology 26:219–241. https://doi.org/10.1111/gcb.14815

Tognetti C, Laos F, Mazzarino MJ, Hernández MT (2005) Composting vs. Vermicomposting: A Comparison of End Product Quality. Compost Science & Utilization 13:6–13. https://doi.org/10.1080/1065657X.2005.10702212

Walvoort D, Brus [aut D, cph, Gruijter J de (2025) Spcosa: Spatial Coverage Sampling and Random Sampling from Compact Geographical Strata

Wickham H (2016) Tidyverse: Easily Install and Load the 'Tidyverse'

Zhang J, Li S, Jiang P, Wang R, Guo J, Xiao H, Wu J, Shaaban M, Li Y, Huang M (2024) Organic fertilizer substituting 20% chemical N increases wheat productivity and soil fertility but reduces soil nitrate-N residue in drought-prone regions. Frontiers in Plant Science 15: https://doi.org/10.3389/fpls.2024.1379485

## 5.9 Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## 5.11 Author contributions

Francesco Serafini: Conceptualization; Investigation; Formal Analysis; Writing – Original Draft Preparation.

Ottorino-Luca Pantani: Methodology; Investigation; Formal Analysis; Writing – Review & Editing.

Margherita Santoni: Methodology; Investigation; Formal Analysis; Writing – Review & Editing.

Marco Nocentini: Investigation; Writing – Review & Editing.

Lorenzo Ferretti: Investigation; Writing – Review & Editing.

Anita Maienza: Validation; Formal Analysis; Writing – Review & Editing.

Gaio Cesare Pacini: Conceptualization; Supervision; Project Administration; Writing – Review & Editing.

All authors have read and approved the final version of the manuscript.

## 5.12 Supplementary Materials

Table S16: Main physico-chemical characteristics of soil from the Montepaldi Long Term Experiment.

| Parameter | Value |
|---|---|
| Sand (%) | 17.6 |
| Silt (%) | 49.4 |
| Clay (%) | 33.0 |
| Texture | Silty clay loam |
| pH ($H_2O$) | 8.3 |
| CEC (cmol$^+$ kg$^{-1}$) | 18.2 |
| Total $P_2O_5$ (%) | 1.66 |
| Available $P_2O_5$ (mg kg$^{-1}$) | 27 |
| Exchangeable $K_2O$ (mg kg$^{-1}$) | 143 |

Table S17: Agronomic details (management, crops, and field operations) at the MoLTE site across two seasons (2023–2024, 2024–2025).

| | 2023–2024 | | 2024–2025 | |
|---|---|---|---|---|
| Management | Old Organic | New Organic | Old Organic | New Organic |
| Previous crop | *Medicago sativa* L. var. Maraviglia | *Medicago sativa* L. var. Maraviglia | *Medicago sativa* L. var. Maraviglia | *Medicago sativa* L. var. Maraviglia |
| Actual crop | *Triticum dicoccum* L. | *Triticum dicoccum* L. | *Triticum aestivum* L. var. Gentilrosso + Andriolo + Inallettabile | *Triticum aestivum* L. var. Gentilrosso + Andriolo + Inallettabile |
| Plant density (kg ha$^{-1}$) | 150 | 150 | 160 | 160 |
| Amendments distribution | 2023-10-15 | 2023-10-15 | 2024-10-09 | 2024-10-09 |
| Plowing | 2023-10-23 | 2023-10-23 | 2024-10-15 | 2024-10-15 |
| Disk harrowing | 2023-12-23 | 2023-12-23 | 2024-11-16 | 2024-11-16 |
| Sowing | 2023-12-28 | 2023-12-28 | 2024-12-06 | 2024-12-06 |
| Harvest | 2024-07-19 | 2024-07-19 | 2025-07-18 | 2025-07-18 |

Table S18: Sampling dates for each indicator during the experiment.

| Indicator | Sampling date |
|---|---|
| **Chemical indicators** | |
| Available P | 2023-10-13; 2024-10-07; 2025-06-03 |
| Soil Organic C | 2023-10-13; 2024-10-07; 2025-06-03 |
| Total N | 2023-10-13; 2024-10-07; 2025-06-03 |
| **Physical indicators** | |
| Aggregate stability | 2023-10-13; 2024-10-07; 2025-06-03 |
| Soil penetration resistance | 2024-05-24 |
| **Biological indicators** | |
| Earthworm | 2024-05-09; 2024-05-15; 2025-04-02; 2025-04-03 |
| **Productivity indicators** | |
| Crop development and physiology | 2024-04-30; 2024-05-10; 2024-05-16; 2024-05-31; 2024-06-07; 2024-06-19; 2025-04-30; 2025-05-07; 2025-05-13; 2025-05-22; 2025-05-27; 2025-06-03; 2025-06-11 |
| Yield | 2024-07-11; 2025-06-30 |

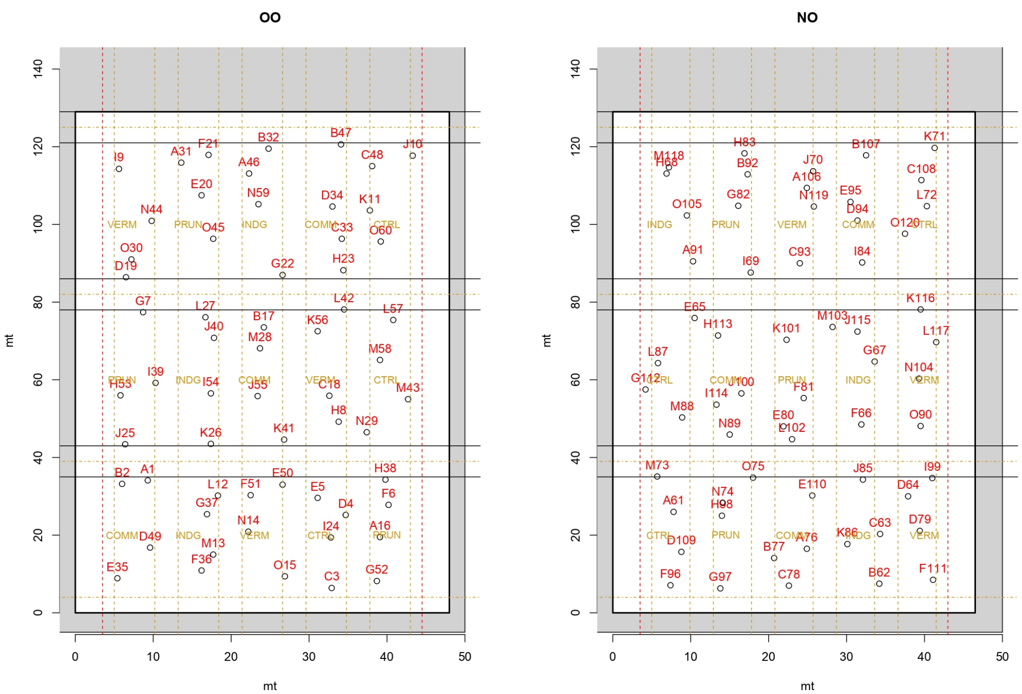


Figure S6: Experimental field layout at the Montepaldi Long-Term Experiment (MoLTE). The two panels represent the Old Organic (OO) and New Organic (NO) management systems. Each system consists of 15 plots (9 m wide × 35 m long), arranged in parallel strips, each plot corresponding to a specific treatment (TRT), indicated by gold labels. Within each plot, four fixed sampling sites (SUB-REP) were established and maintained at the same spatial positions throughout the entire duration of the experiment. Plot boundaries are indicated by solid black lines, dashed internal grids indicate the spatial reference used to locate sampling points, and red labels identify individual sampling sites.

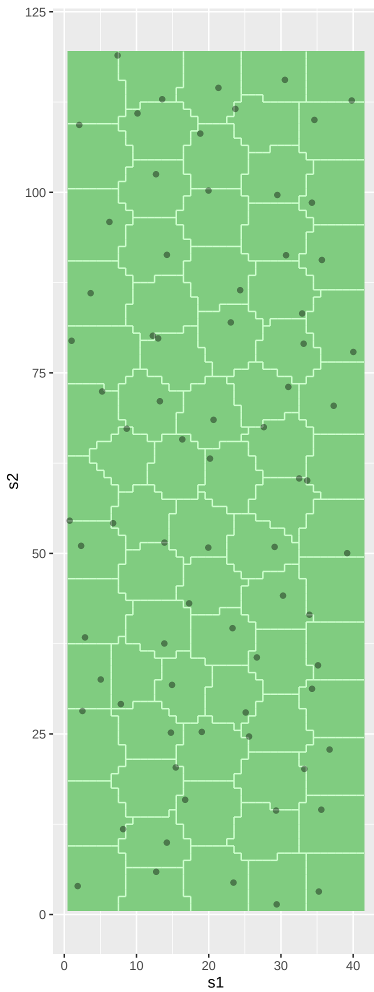


Figure S7: Spatial distribution and randomization of sampling sites within plots. Each polygon represents a sampling area of identical size corresponding to a SUB-REP. Within each area, the sampling location is defined by randomized x–y coordinates, generated once and kept constant for repeated measurements over time. Points indicate the actual sampling positions used in the field. This approach ensured spatial independence among SUB-REP while preserving temporal consistency.

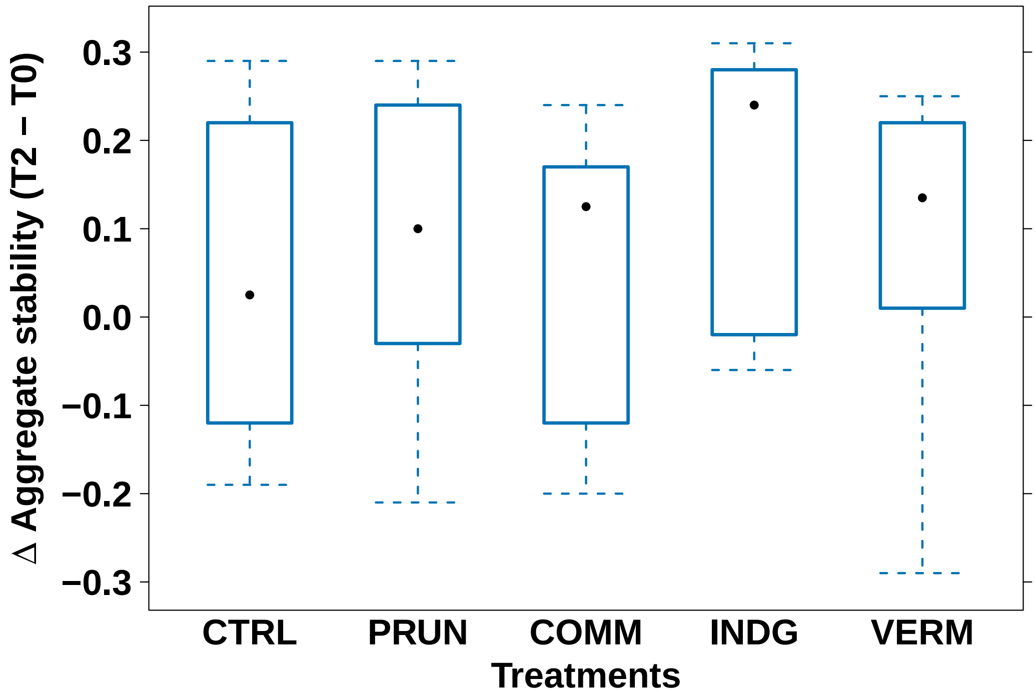


Figure S8: Change (Δ) in aggregate stability (T2 - T0) across treatments.

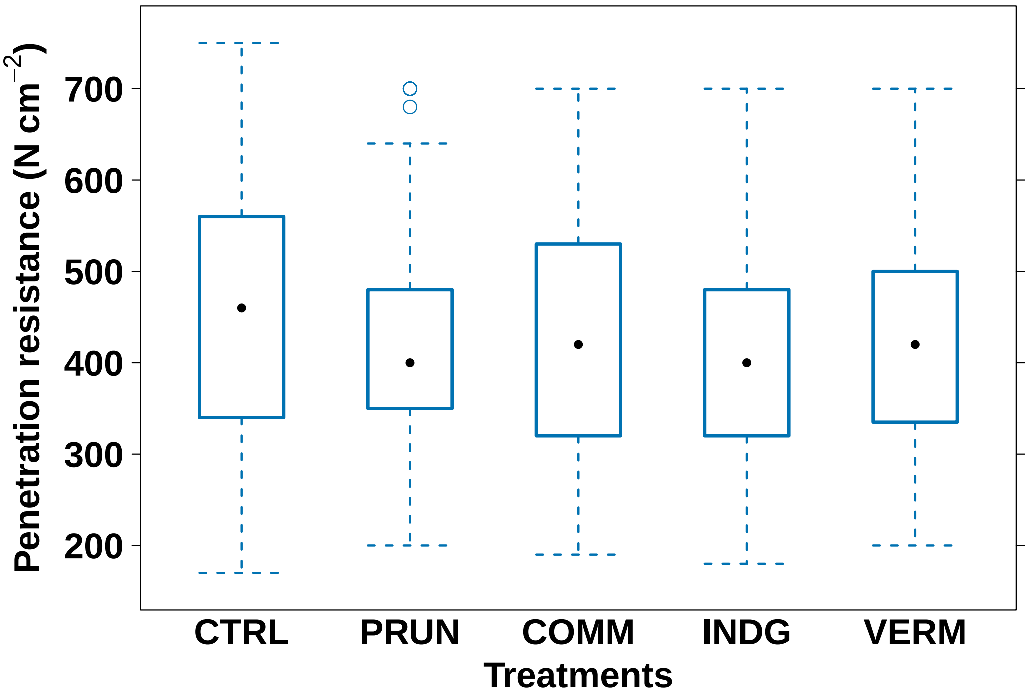


Figure S9: Soil penetration resistance(N $cm^{-2}$) across treatments.

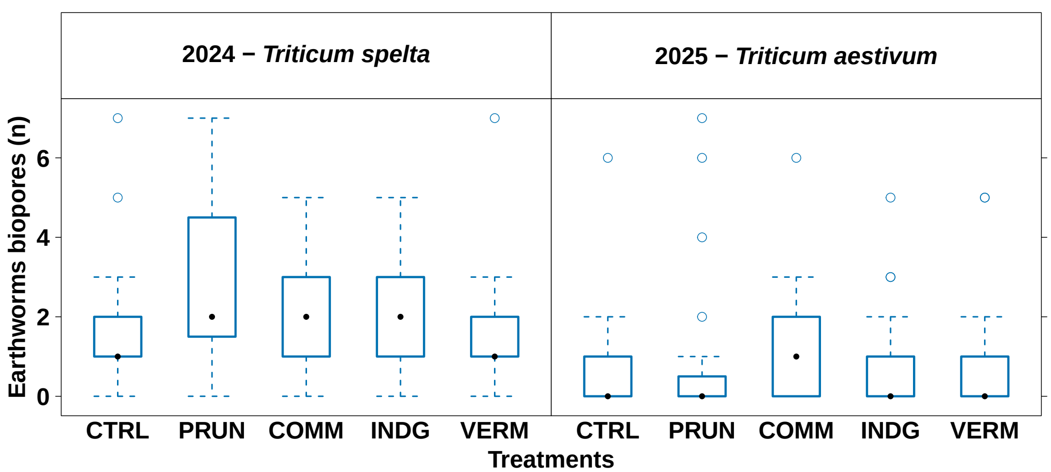


Figure S10: Number of visible earthworm biopores by treatment in 2024 - Triticum spelta and 2025 - Triticum aestivum.

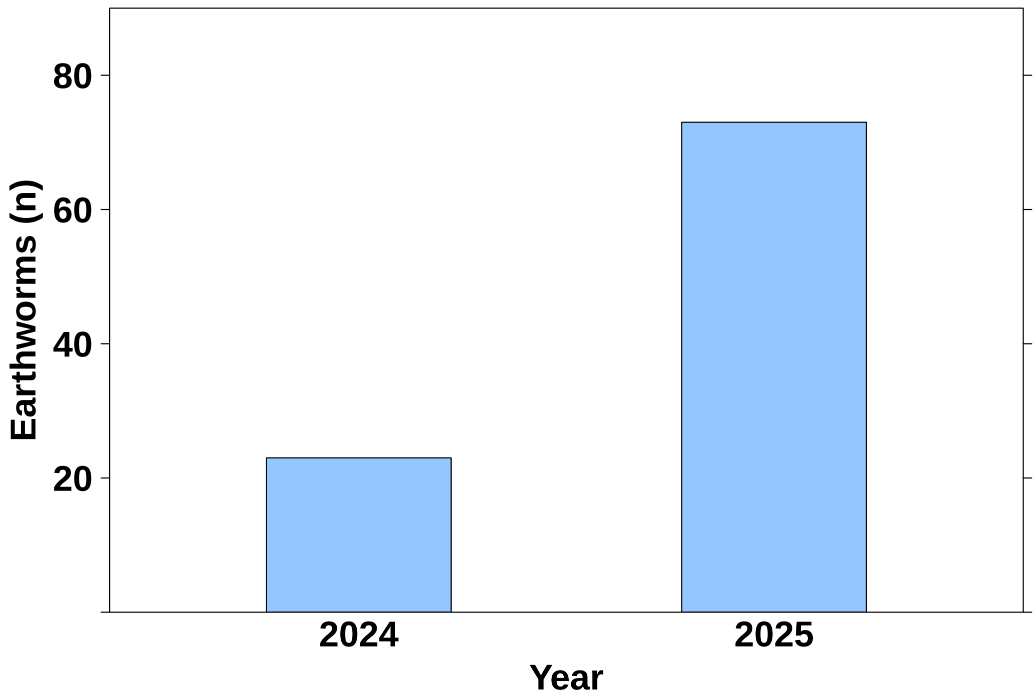


Figure S11: Field abundance of earthworms by year.

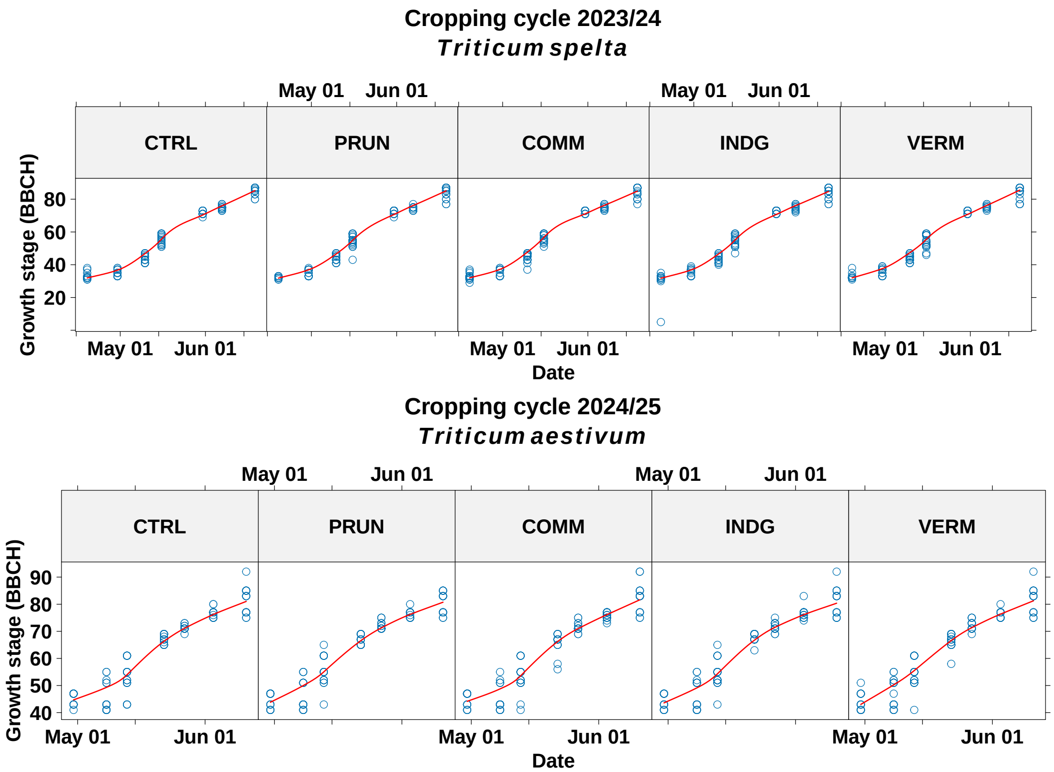


Figure S12: Crop phenology (BBCH growth stage) over time by treatment for the two cropping cycles (2023/24 - Triticum spelta; 2024/25 - Triticum aestivum).

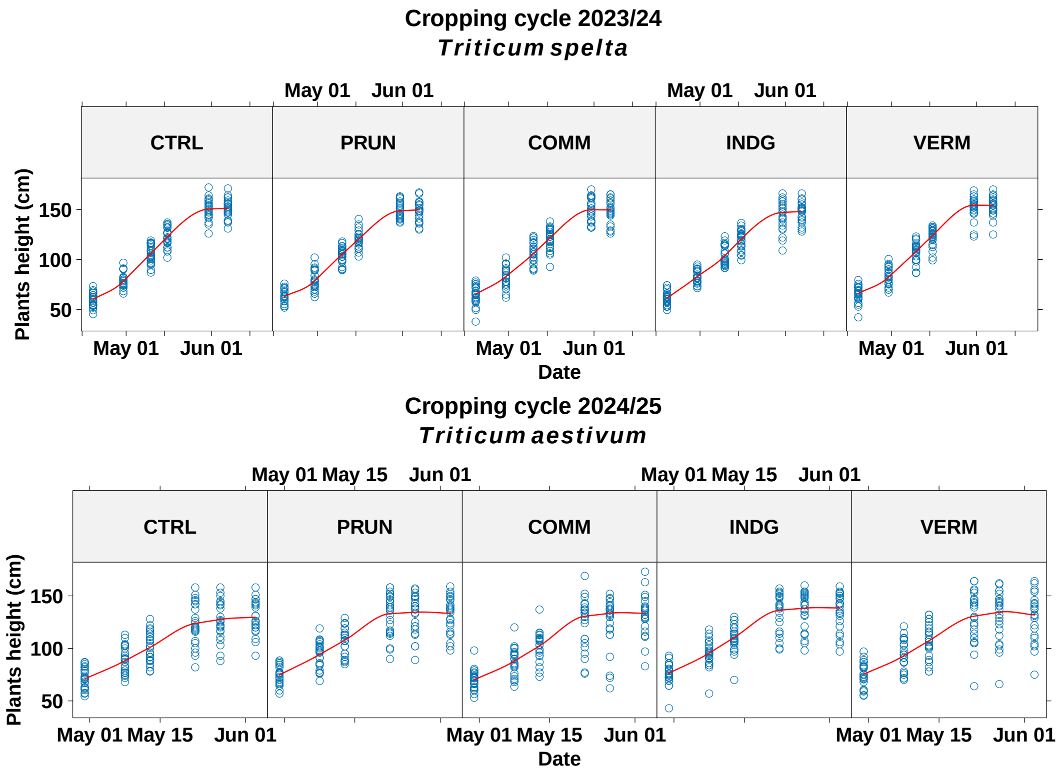


Figure S13: Plant height (cm) over time by treatment for the two cropping cycles (2023/24 - Triticum spelta; 2024/25 - Triticum aestivum).

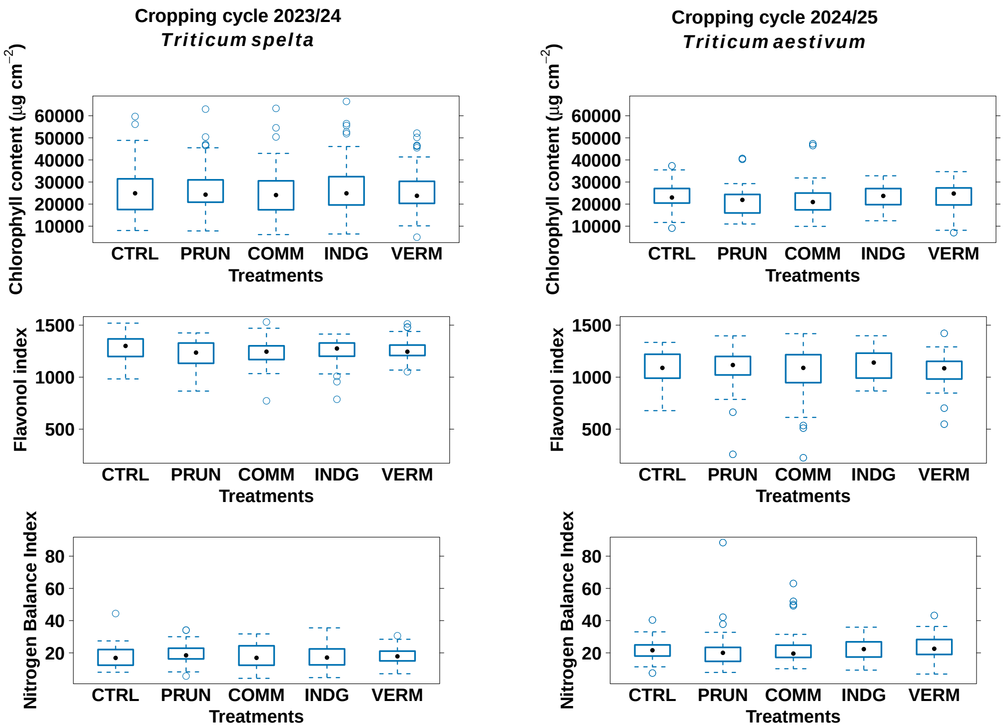


Figure S14: Leaf optical traits measured with Dualex: chlorophyll content (µg $cm^{-2}$), flavonol index, and Nitrogen Balance Index (NBI) by treatment in 2023/24 - Triticum spelta and 2024/25 - Triticum aestivum.

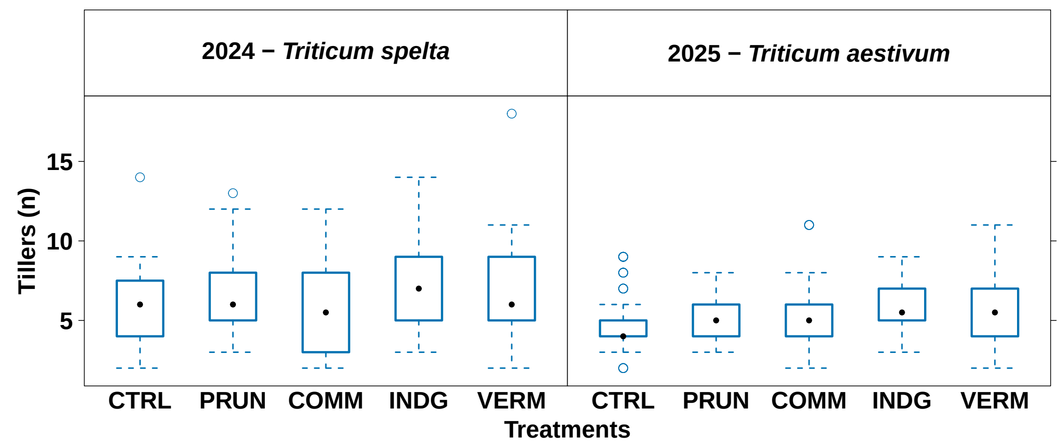


Figure S15: Tillers per plant by treatment in 2024 - Triticum spelta and 2025 - Triticum aestivum.

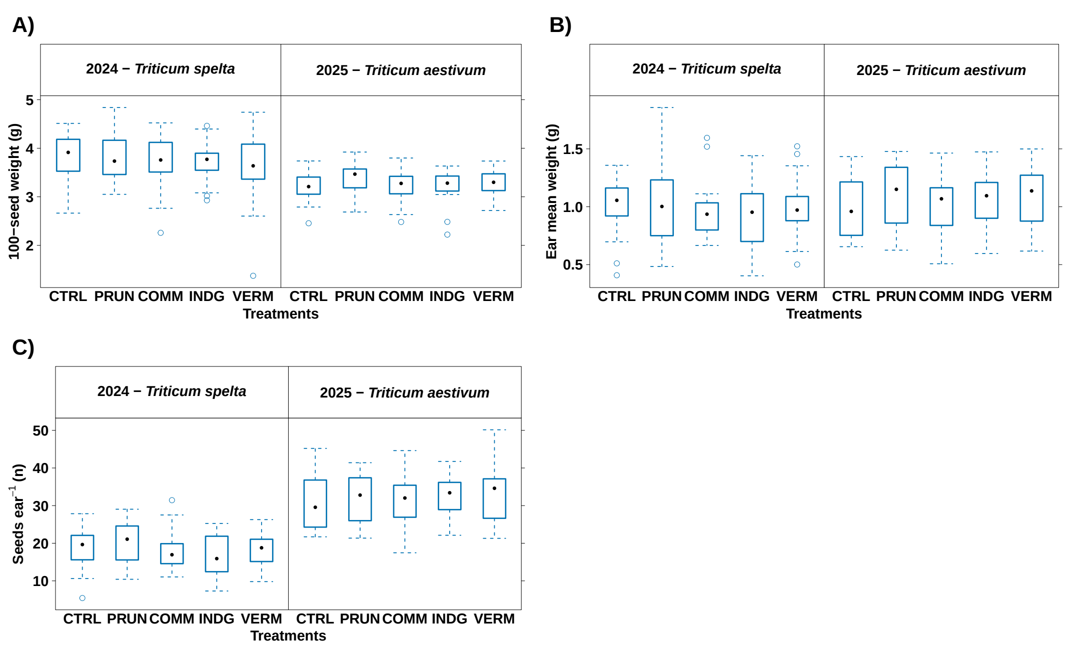


Figure S16: Yield components by treatment for 2024 - Triticum spelta and 2025 - Triticum aestivum: A) 100-seed weight (g), B) mean spike weight (g spike$^{-1}$), and C) seeds per spike (n).

# 6 Behavioral and Functional Responses of *Hormogaster samnitica* to Different Organic Amendments

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## 6.1 Abstract

The decline of soil organic matter in Mediterranean agroecosystems, coupled with the under-utilization of crop residues, calls for sustainable strategies that enhance soil health while valorizing local organic resources. This study investigates the behavioral and functional responses of *Hormogaster samnitica*, a large-bodied, endogeic earthworm, to four organic amendments derived from agricultural residues: vine pruning compost (PRUN), vine pruning compost with a commercial accelerator (COMM), vine pruning compost inoculated with Indigenous Microorganisms (INDG), and vermicompost from spent coffee grounds (VERM). The experiment was conducted in laboratory mesocosms using repacked soil columns. Earthworms were introduced at three body size classes ("big", "medium" and "small"), and their impact on soil porosity and infiltration was assessed via X-ray tomography and infiltration tests. Results showed that body size significantly affected earthworm activity, with larger individuals creating wider and deeper burrows and markedly increasing water infiltration. Among treatments, COMM significantly enhanced macroporosity in the amended soil layer and induced a marked shift in the vertical distribution of macropores. However, despite increased porosity, compost-based amendments generally reduced infiltration rates compared to the CTRL. VERM showed limited effects on both bioturbation and infiltration. A two-choice preference test revealed that *H. samnitica* exhibited weak substrate preferences, while the more generalist *Aporrectodea caliginosa* clearly favored compost-based treatments over VERM and CTRL. Overall, these findings highlight the importance of considering species-specific traits and amendment properties when evaluating the ecological impact of organic inputs, and support the use of compost as a biostimulant for soil fauna and structure enhancement in Mediterranean systems.

## 6.2 Keywords

Agroecology, Soil amendments, Earthworms, Burrowing behaviour, Soil bioturbation, Tomography

## 6.3 Highlights

- *Hormogaster samnitica* responses to four organic amendments were evaluated
- Earthworm body size influenced burrow depth and soil water infiltration
- Vine pruning compost enhanced earthworm activity but reduced infiltration rates
- Vermicompost had limited effects on bioturbation and hydraulic properties
- Earthworm substrate preference was species-specific and amendment-dependent

## 6.4 Introduction

Agricultural intensification and the separation between crop production and livestock farming have markedly reduced the return of organic materials to cultivated soils (Manlay et al. 2007). This transformation has played a major role in the steady decline of soil organic matter (SOM), a fundamental component of soil fertility and the long-term functioning of agroecosystems (Garrett et al. 2020). In the past, SOM levels were maintained through the regular application of farm-generated inputs such as manure and plant residues. With the reduction of mixed farming systems and a growing dependence on synthetic fertilizers, these traditional nutrient cycles have been increasingly disrupted, particularly in arable systems that no longer benefit from livestock-based organic inputs (Canali et al. 2005; Feller et al. 2012). As a result, many Mediterranean soils now face a SOM deficit, with consequences that include reduced structural stability, lower water-holding capacity, diminished microbial activity, and a general decline in long-term productivity (Ferreira et al. 2022).

At the same time, modern agriculture generates vast quantities of plant-based residues that are often underutilized or poorly managed, representing a missed opportunity for SOM replenishment. Crop residues alone account for a substantial portion of this organic waste, with global annual production estimated at around 74 Tg of dry matter, including approximately 45 Tg derived from rice and wheat cultivation (Medina et al. 2015). Vineyard prunings, for instance, represent a considerable biomass flow in viticultural regions but are commonly disposed of by burning or left to decay unmanaged. These practices not only waste a potentially valuable resource but also contribute to atmospheric pollution and greenhouse gas emissions (Lou and Nair 2009). Addressing the dual challenge of SOM depletion and residue management therefore requires innovative strategies that are both agronomically effective and environmentally sound (Kanwal 2021).

Within this context, the principles of circular economy and agroecology offer promising pathways (FAO 2018). Agroecological approaches emphasize the recycling of nutrients and organic matter, the reduction of external inputs, and the valorization of on-farm resources, aiming to build resilient and self-sustaining agroecosystems. Circular economy strategies, likewise, promote the transformation of waste streams into productive inputs, aligning resource use efficiency with environmental stewardship (Vásquez Neyra et al. 2025).

Earthworms play a pivotal role in shaping soil structure, porosity, and water dynamics. Through their burrowing and feeding activities, they create macropores, redistribute organic matter, and stimulate microbial activity, thereby enhancing soil function and fertility. As ecosystem engineers, earthworms contribute to several key soil processes, including aeration, infiltration, and aggregation (Bertrand et al. 2015).Their response to organic amendments is therefore an indicator of amendment quality and a determinant of amendment efficacy. These amendments are applied to increase or restore the soil fertility and earthworms are important bioagents that will contribute directly or indirectly to their decomposition through their burial ingestion and translocation within the soil. Thus, earthworms' behavioral and functional traits (e.g., burrowing, casting, avoidance) are strongly linked to the physical processes of bioturbation that support their ecological role (Capowiez et al. 2024; Lowe and Butt 2002). While the behavior of widely studied endogeic or anecic species such as *Aporrectodea caliginosa* and *Lumbricus terrestris* has been extensively investigated, much less is known about the functional traits of endogeic species native to Mediterranean soils. Previous studies have shown that *A. caliginosa* and *L. terrestris* have different responses mainly linked to the location of these amendments (Frazão et al. 2019): amendments on or close to the surface benefited the anecics and those mixed with the soil promoted the endogeics. Beyond this typical responses, amendments are also able to modify the burrowing behavior of earthworms of both ecological categories (Ducasse et al. 2025; Frøseth et al. 2014; Mombo et al. 2018).

*Hormogaster samnitica*, a large-bodied, deep-burrowing earthworm endemic to the Mediterranean region (Omodeo and Rota 2008)and commonly associated with calcareous, clay-rich soils typical of Central Italy, remains virtually unstudied despite its ecological relevance (Marchán et al. 2018). Adult individuals can reach up to 20 g in body mass. Unlike more mobile and generalist species, *H. samnitica* is characterized by low dispersal ability, low reproductive rates, and a long lifespan, traits typically associated with stable and weakly disturbed soil environments. For these reasons, this species represents a suitable biological model to investigate soil functional responses under low-disturbance agroecological management. However, to date, no studies have systematically characterized its bioturbation behavior or its responses to organic amendments under controlled conditions. According to Bouché (Bouché 1972), *H. samnitica* was classified as endogeic based on pigmentation traits, although its close phylogenetic affinity with the more pigmented *Hormogaster praetiosa*, described as anecic, suggests functional traits that remain insufficiently resolved.

The present study aims to fill these knowledge gaps by evaluating the short-term effects of different organic amendments on the behavior and functional impact of *H. samnitica*, thereby contributing to its functional characterization within the existing ecological categories. The first objective of the experiment was to characterize the bioturbation activity of *H. samnitica* individuals depending on their different body sizes (classified into small, medium, and big), to determine whether weight class influences key outcomes such as cast production, burrow formation, and soil infiltration.

The second objective was to evaluate the effects of the four organic amendments on earthworm activity (as indicated by weight change and macroporosity produced) and to assess how this activity influences key soil physical indicators, such as water infiltration.

We also included a two-choice preference test to assess whether *H. samnitica* and a more generalist species, *A. caliginosa*, exhibit differential attraction to the various amendments. This behavioral assay aimed to reveal short-term preferences that may correlate with amendment quality and inform future field-scale applications.

Accordingly, we hypothesized that: (i) larger *H. samnitica* individuals would have a greater influence on soil structure and function than smaller one; (ii) the addition of organic amendments would stimulate earthworm activity and modify burrowing patterns compared to unamended soil; and (iii) earthworms would display clear substrate preferences for fresh organic amendments and less to vermicompost that was already processed by earthworms (*Eisenia fetida*).

Overall, this study provides a first characterization of *H. samnitica* role in the soil under controlled conditions and offers new insights into how different composting strategies influence earthworm activity and soil physical properties. By focusing on locally sourced amendments and a native species, the research contributes to the development of context-specific, sustainable soil management practices in Mediterranean farming systems. It also underscores the importance of integrating soil fauna into the evaluation of organic amendments, not only as bioindicators but also as key agents of soil restoration.

## 6.5 Material and Methods

### 6.5.1 Earthworms collecting

*Hormogaster samnitica* individuals used in this experiment were collected in January 2025 from the Montepaldi Long-Term Experiment (MoLTE), located in San Casciano Val di Pesa (FI, Italy; 11°09 08 E, 43°40 16 N, 90 m a.s.l.). Earthworms were sampled by digging the soil with a shovel to a depth of approximately 30 cm and hand-sorting the excavated soil. This species is endemic to the Mediterranean region and represents the dominant earthworm taxon in the Montepaldi area, accounting for more than 80% of the total earthworm population, as previously documented for this site (Pantani et al. 2022). Its high local abundance reflects the biogeographical distribution of *H. samnitica* and its adaptation to the soil properties and pedoclimatic conditions characteristic of the study area. After collection, earthworms were gently washed, weighed, and classified into three body-mass classes: "big" (>4 g, up to 12.79 g), "medium" (2–4 g), and "small" (<2 g, down to 1.07 g). Individuals in the "big" class were either adults or subadults, identified by the presence of a developed puberculum.

*A. caliginosa* adults used for the preference test were collected by hand-sorting the soil in April 2025 from a humid pasture close to Avignon. Their mean weight was 0.41 g.

### 6.5.2 Organic amendment treatments

The experiment included four organic amendment treatments (PRUN, COMM, INDG and VERM) and one unamended control (CTRL). Three of the organic amendments were derived from vine prunings collected at the organic farm Castello del Trebbio (Pontassieve, Florence, Italy). Prunings were mixed in a 1:1 (w/w) ratio with alfalfa, shredded, and composted near the fields of the Montepaldi Long-Term Experiment. In detail, the PRUN treatment consisted of composted vine prunings without additives. The COMM treatment included vine prunings composted with 600 g $m^{-3}$ of a commercial composting accelerator. The INDG treatment involved composting vine prunings inoculated with Indigenous Microorganisms ($IMO_2$), following Korean Natural Farming techniques. The $IMO_2$ culture was prepared through two stages, $IMO_1$ (collection) and $IMO_2$ (propagation), and applied to the compost pile at a rate of 500 g diluted in 10 L of water. All compost piles were equipped with three internal data loggers and were regularly turned and moistened to maintain aerobic conditions and enhance microbial activity. The VERM treatment consisted of vermicompost produced from spent coffee grounds and was provided by Funghi Espresso Società Agricola s.r.l. Due to these different origins, VERM has different properties compared to the 3 other amendments.

Physico-chemical properties of the four amendments are summarized in Table 3.

Table 3: Averages (± standard deviation) of the physico-chemical qualities of the organic amendments, determined according to internationally recognized standard procedures (ISO, TMECC) (n = 3).

| Treatment | Density (kg $L^{-1}$) | Moisture (%) | pH | EC (mS $cm^{-1}$) | C (%) | N (%) | C/N |
|---|---|---|---|---|---|---|---|
| PRUN | 0.13 | 13.2 | 6.9 | 0.90 | 43.9 | 0.80 | 57.4 |
| | (0.01) | (0.25) | (0.05) | (0.36) | (0.67) | (0.03) | (3.39) |
| COMM | 0.13 | 13.5 | 6.9 | 0.80 | 44.5 | 0.80 | 58.0 |
| | (0.01) | (0.40) | (0.02) | (0.02) | (0.24) | (0.02) | (1.61) |
| INDG | 0.15 | 12.8 | 7.0 | 0.70 | 43.6 | 0.70 | 62.6 |
| | (0.01) | (0.06) | (0.01) | (0.02) | (1.34) | (0.05) | (3.03) |
| VERM | 0.49 | 67.9 | 5.8 | 0.90 | 42.8 | 5.00 | 8.5 |
| | (0.01) | (0.78) | (0.05) | (0.06) | (1.53) | (0.19) | (0.03) |

### 6.5.3 Mesocosms and experimental design

To evaluate the impact of the different products on earthworm activities, we realized mesocosms in PVC columns (30 cm depth and 16 cm diameter) with a mesh (2 mm) glued at the bottom. The mesocosms were built with soil taken from an orchard since more than 15 years in Montfavet, near Avignon (43°55' N, 4°48' E) in the SE of France.

The soil was sieved to 2 mm. For each layer, we used 650 g (± 1 g) of soil with an average moisture content of 16%, and pressed it using a hydraulic press between 160 and 170 kPa for 3 min to obtain an average density of 1.2 g $cm^{-3}$. This operation was repeated 12 times to obtain 26 cm depth columns.

Amendmentss were added in the last five layers (0-10 cm depth) close to the surface. The quantity of each amendment was calculated based on an application rate equivalent to 32 t $ha^{-1}$ FW, i.e. with the same rate than when these products were applied on the field. The products were homogeneously mixed with the soil. Ten soil columns were prepared for each of the five treatments. For each treatment, five columns were inoculated with one *H. samnitica* individual belonging to the "big" weight class and five with one individual from the "medium" class. Five columns were established without amendments and without earthworms as control cores. Additionally, to gain insights into the ecology of the different life stages of this species, five columns of the CTRL treatment were set up using individuals from the "small" weight class. In total, the experiment included 60 soil columns. Each earthworm was weighed prior to its introduction into the corresponding mesocosm.

The columns were left for 1 month in an outside shelter (with temperatures ranging from 13 to 18°C during the experiment). During this period, surface casts were collected by hands every two days, oven-dried, and weighed.

### 6.5.4 Monitoring of the soil porosity by X-ray tomography and 3D reconstruction of burrows

The soil cores were analyzed by X-ray tomography with a medical scanner in the INRAE centre of Nancy. The settings of the scanner were 120 kV and 50 mA. The images were then transformed into 8-bit images using ImageJ and the following parameters: -1000 for WW and 2000 for WL. The porosity created by the earthworms was obtained by binarizing the images wit ha fixed threshold (greylevel=200) since the peaks for porosity and soil matrix were well separated (Capowiez et al. 1998). he macropores were characterized by computing the diameter, the volume of porosity (total and in two layers) and the barycenter. Diameter was estimated by the median of the the most circular pores in the 2D images (circularity, as defined by ImageJ, $> 0.8$). The volume of macroporosity was the sum of the burrows whose volume was greater than 30 voxels. The barycenter was computed as the vertical barycenter of all the burrows (taking their volume into account).

The macroporosity data were aggregated into two depth layers: the upper layer (H1, 0–10 cm), corresponding to the first five scanned sections where organic amendments were applied, and the lower layer (H2, 10–26 cm), comprising the remaining seven sections. This stratification allowed the evaluation of amendment-induced effects on burrow formation near the surface as well as their propagation into deeper soil layers.

### 6.5.5 Water infiltration experiment and earthworm weight

ollowing the tomographic analysis, infiltration tests were conducted using a single-ring infiltration method, also known as the simplified 'Beerkan' approach (Capowiez et al. 2015). Each core was placed on a support that allowed free drainage, and a fixed water volume (140 mL, equivalent to a water layer of 0.7 cm) was poured onto the soil surface at time zero. The time required for the complete infiltration of this volume was recorded. When the first volume had infiltrated completely, a second fixed volume of water was added. This process was repeated sequentially for a total of eight identical water volumes. To estimate the final infiltration rate (expressed in mm min$^{-1}$), we performed a linear regression of cumulative infiltration versus elapsed time. It is important to highlight that this method employed primarily for comparative purposes and to simulate intense rainfall events.

At the end of the infiltration test, each mesocosm was carefully dismantled, and the surviving earthworms were recovered and weighed to assess weight changes during the experiment.

### 6.5.6 Preference test

A two-choice preference test was carried out using plastic boxes (26 × 28.4 × 8.5 cm) filled with 1 kg of sieved soil (1 cm depth). Each box was divided into two equal halves, each receiving a different treatment. All possible pairwise combinations of the five treatments (CTRL, PRUN, COMM, INDG, VERM; 10 combinations) were tested, with three replicates per combination. Organic amendments were incorporated into the soil at a rate equivalent to 32 t ha$^{-1}$ FW. After amendment incorporation, earthworms were placed at the center of the box and allowed to move freely between the two soil compartments.

Two earthworm species were tested: *H. samnitica* (8 "small" individuals per box, chosen as they could move easily in the boxes and were available in sufficient numbers) and adults of *Aporrectodea caliginosa* (10 individuals per box). The boxes were kept in darkness at 18 °C for 60 hours.

At the end of the incubation period, the soil was carefully hand-sorted and the number of individuals present in each compartment was recorded. The number of earthworms recovered on each side of the box was used as the response variable in a binomial generalized linear model, from which the probability of choosing each treatment was estimated.

### 6.5.7 Statistical analysis

All statistical analyses were performed in R version 4.5.0 (R Core Team 2023), and data visualization was conducted using the lattice (Sarkar 2001) and ggplot2 (Wickham 2011) packages. Data preprocessing and merging were handled through dplyr (Wickham et al. 2021) and tidyverse (Wickham 2023) functions. Linear models (LM) were employed to evaluate the effects of treatments and earthworm weight classes on continuous variables. The assumptions of normality and homoscedasticity were checked by visual inspection of Q–Q plots and residual vs. fitted plots, respectively. When heteroscedasticity was detected, data were log-transformed.

Infiltration dynamics were assessed by estimating linear trends (slopes) of water infiltration over time, expressed in mm min$^{-1}$ (Capowiez et al. 2015). These trends were extracted using the emtrends function from the emmeans package (Lenth 2017).

For the preference tests, the observed number of earthworms recovered on each side of the box was used as the response variable in a binomial generalized linear model (GLM), with treatment as the explanatory factor. Model results were expressed on the response scale as estimated probabilities of soil side choice. Post-hoc comparisons among treatments were conducted using Tukey-adjusted grouping letters (Lenth 2017; McCullagh 2019).

## 6.6 Results

### 6.6.1 Effects of the earthworms of different weight classes

All subsequent analyses presented in this section refer exclusively to the unamended control (CTRL) mesocosms, in which no organic amendment was applied. This allowed us to isolate the effect of *H. samnitica* body weight on soil structural properties and biological activity, independently of amendment-induced variability.

Cast production was observed exclusively during the first 48 hours following the introduction of *H. samnitica* individuals into the soil columns. No surface activity or additional cast deposition occurred during the remainder of the one-month incubation period. Casts were recorded only in some mesocosms containing "big" (>4 g) and "medium" (2–4 g) individuals, while no surface casts were observed in the presence of "small" earthworms (<2 g).

Weight change differed among weight classes, with higher values in the "big" and "medium" groups than in the "small" group. In particular, the "small" class showed significantly lower weight change than the "big" class (p = 0.0125).

Table 4: Mean values (± standard deviation) of selected parameters measured in mesocosms containing individual earthworms of different weight classes. Different letters within rows indicate statistically significant differences between weight classes based on Tukey-adjusted post-hoc comparisons ($p < 0.05$).

| Parameter | Big (> 4 g) | Medium (2 − 4 g) | Small (< 2 g) |
|---|---|---|---|
| Weight change (%) | 33.0 (11.7) $^{b}$ | 17.0 (18.9) $^{ab}$ | 3.73 (15.9) $^{a}$ |
| Macropore diameter (mm) | 6.84 (1.160) $^{b}$ | 5.94 (0.579) $^{b}$ | 3.80 (0.699) $^{a}$ |
| Macropore volume H1 layer ($cm^3$) | 2.47 (1.91) $^{a}$ | 1.24 (0.80) $^{a}$ | 1.60 (0.84) $^{a}$ |
| Macropore volume H2 layer ($cm^3$) | 2.48 (2.00) $^{a}$ | 1.32 (0.51) $^{a}$ | 0.75 (0.36) $^{a}$ |
| Total macroporosity ($cm^3$) | 38.7 (25.1) $^{a}$ | 20.3 (4.69) $^{a}$ | 18.4 (5.46) $^{a}$ |
| Macroporosity barycenter (mm) | 90.2 (30.6) $^{a}$ | 102.0 (19.7) $^{a}$ | 86.2 (21.9) $^{a}$ |
| Infiltration rate (mm $min^{-1}$) | 5.04 (0.754) $^{b}$ | 5.04 (1.11) $^{b}$ | 0.199 (0.101) $^{a}$ |

The structural characteristics of the macropore system varied across earthworm weight classes (Table 4). Individuals in the "big" class created significantly wider burrows (6.84 mm), while the "small" class exhibited markedly narrower macropores (3.8 mm). The difference in diameter between "small" and "big" individuals was statistically significant (p = 0.008), whereas the reduction observed in the "medium" class (5.94 mm) was not significant.

No significant differences among weight classes were detected for macropore volume in either the upper H1 layer or the deeper H2 layer, nor for total macroporosity or the barycenter of macroporosity.

Beyond weight class based comparisons, a positive linear relationship was observed between individual earthworm weight (both before and after incubation) and the total burrow volume (Fig. S21), suggesting that heavier and bigger individuals create more extensive burrow systems.

Water infiltration rates varied markedly among earthworm weight classes (Table 4). Individuals in the “big” and “medium” classes exhibited similarly high infiltration rates (both $5.04\,\mathrm{mm\,min^{-1}}$), while the “small” class showed a dramatically reduced rate ($0.199\,\mathrm{mm\,min^{-1}}$). The rate measured in mesocosms containing “big” individuals was significantly higher than that associated with the “small” class ($p < 0.0001$), highlighting the greater capacity of larger individuals to influence soil hydraulic properties.

### 6.6.2 Effects of the different organic amendments

The relative weight change of H. samnitica individuals varied among organic amendment treatments (Fig. 18; Table S20).

Based on the linear model fitted on percentage weight change, only the COMM treatment resulted in a significantly higher weight gain compared to the unamended control (CTRL), with an estimated increase of +22.8 percentage points. In contrast, no statistically significant differences in weight change were detected between the control and the PRUN, INDG, or VERM treatments.

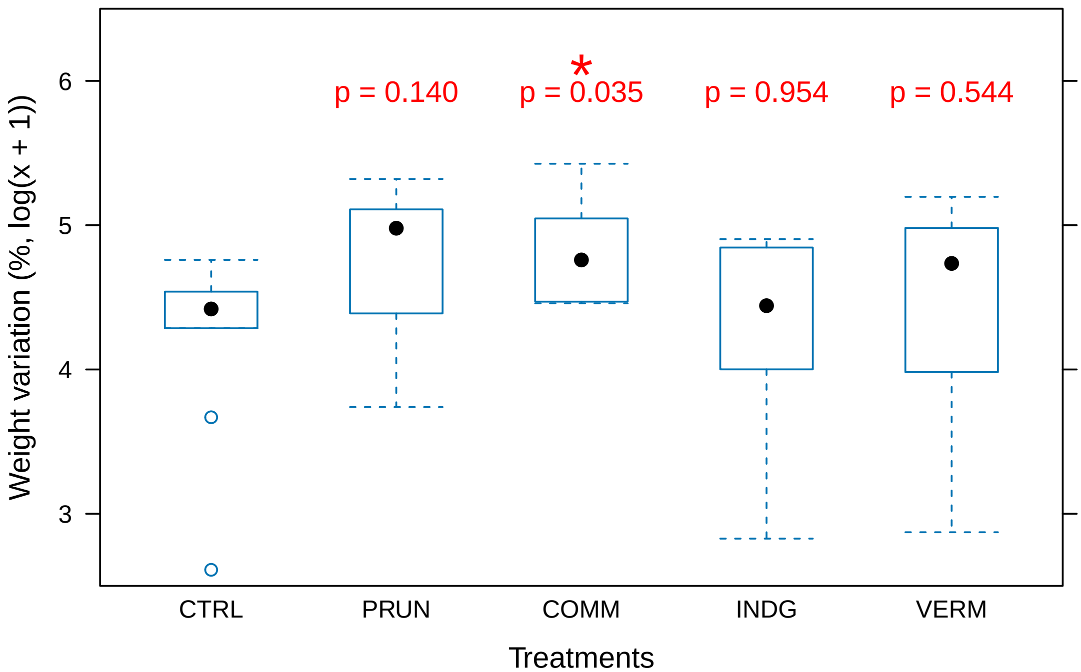


Figure 18: Weight variation (%) of *Hormogaster samnitica* individuals across different organic amendment treatmens. Asterisks indicate statistically significant differences from the control treatment (CTRL): $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

The structure and distribution of macropores were markedly influenced by the type of organic amendment applied. In the upper soil layer (H1, 0–10 cm), where amendments were incorporated, a significant treatment effect on macroporosity was observed (Fig. 19A). The COMM treatment led to the highest increase in macropore volume (+6.2 $cm^3$) compared to the control (CTRL), followed by INDG (+3.5 $cm^3$) and PRUN (+3.1 $cm^3$). These three treatments all differed significantly from the CTRL, while the VERM treatment did not induce a statistically significant change in volume of macroporosity in the H1 layer.

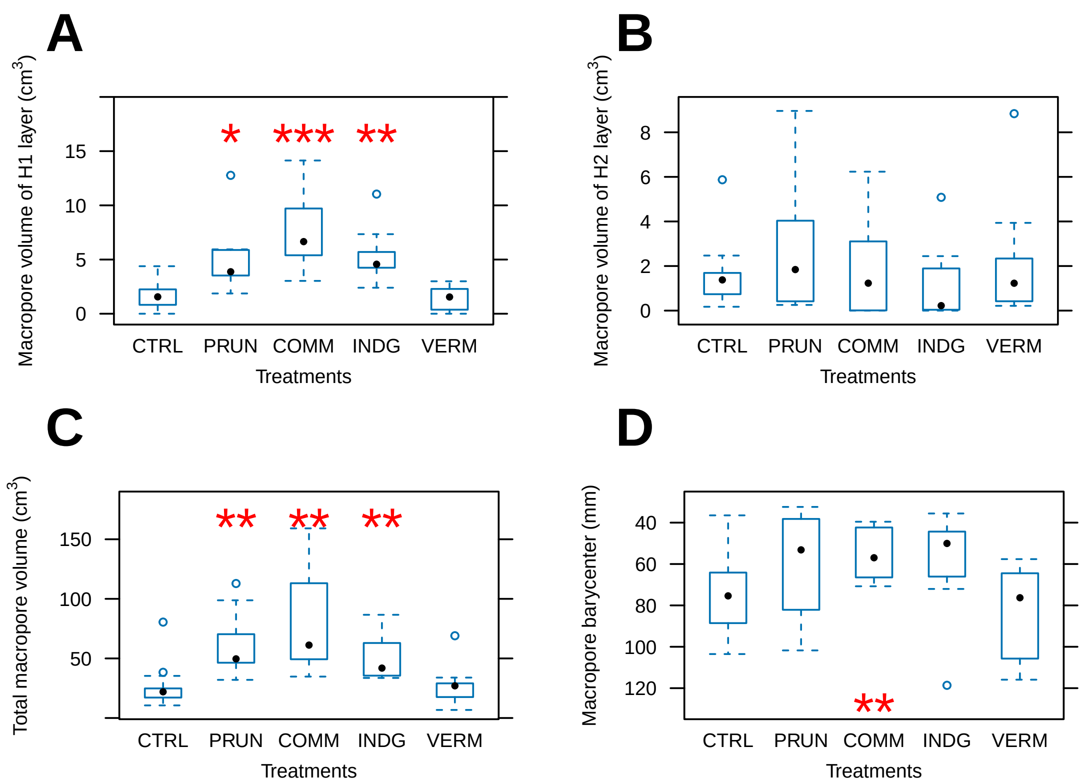


Figure 19: Effects of organic amendments on macropore characteristics. (A) Macropore volume in the H1 layer (0–10 cm), (B) Macropore volume in the H2 layer (10–26 cm), (C) Total macropore volume across the entire column (0–26 cm), (D) Vertical distribution of macroporosity (barycenter). Asterisks indicate statistical significance of pairwise comparisons versus CTRL: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

In the lower layer (H2, 10–26 cm), no significant differences in macroporosity were detected among treatments (Fig. 19B).

When considering the total macropore volume across the entire soil column, the compost-based treatments COMM, PRUN, and INDG significantly increased the total macroporosity relative to the control (Fig. 19C). In contrast, VERM had no appreciable effect.

In addition to changes in volume, the vertical distribution of macropores was also affected (Fig. 19D). The barycenter of macroporosity in the COMM treatment was significantly shifted towards the surface (-23.4 mm), indicating a concentration of macropore formation in the uppermost part of the soil column and a more shallow burrow system. No significant shifts in barycenter position were observed for the other treatments.

The analysis of infiltration trends revealed that all compost-based treatments showed significantly lower infiltration rates compared to the CTRL group (Fig. 20). Specifically, the slope of the infiltration curve in CTRL (5.04 mm min$^{-1}$) was significantly higher than in PRUN (p $< 0.01$), COMM (p $< 0.001$), and INDG (p $< 0.001$), indicating slower infiltration in these amended soils. No significant difference was found between CTRL and VERM (p $= 0.85$), suggesting that the infiltration performance of VERM-treated cores was comparable to the unamended control.

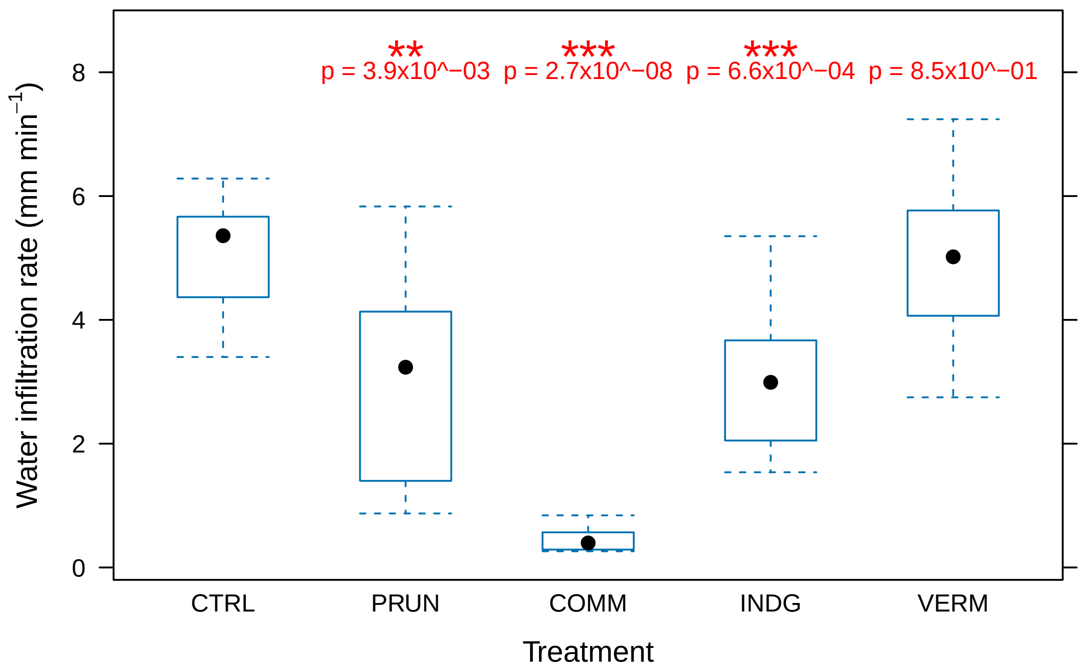


Figure 20: Water infiltration rate (mm min$^{-1}$) measured in soil columns under different organic amendment treatments. Asterisks denote statistically significant differences from the control based on pairwise comparisons: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

### 6.6.3 Preference test

In the two-choice preference test, the distribution of individuals between the two sides of the boxes differed between species and treatments. Based on the estimated probabilities derived from the binomial model, *A. caliginosa* showed a clear preference for COMM, followed by INDG and PRUN, all significantly higher than CTRL. In contrast, *H. samnitica* exhibited weaker treatment differentiation. Although PRUN showed the highest estimated probability for *H. samnitica*, overlapping grouping letters indicate that no significant preference pattern emerged (Fig. 21).

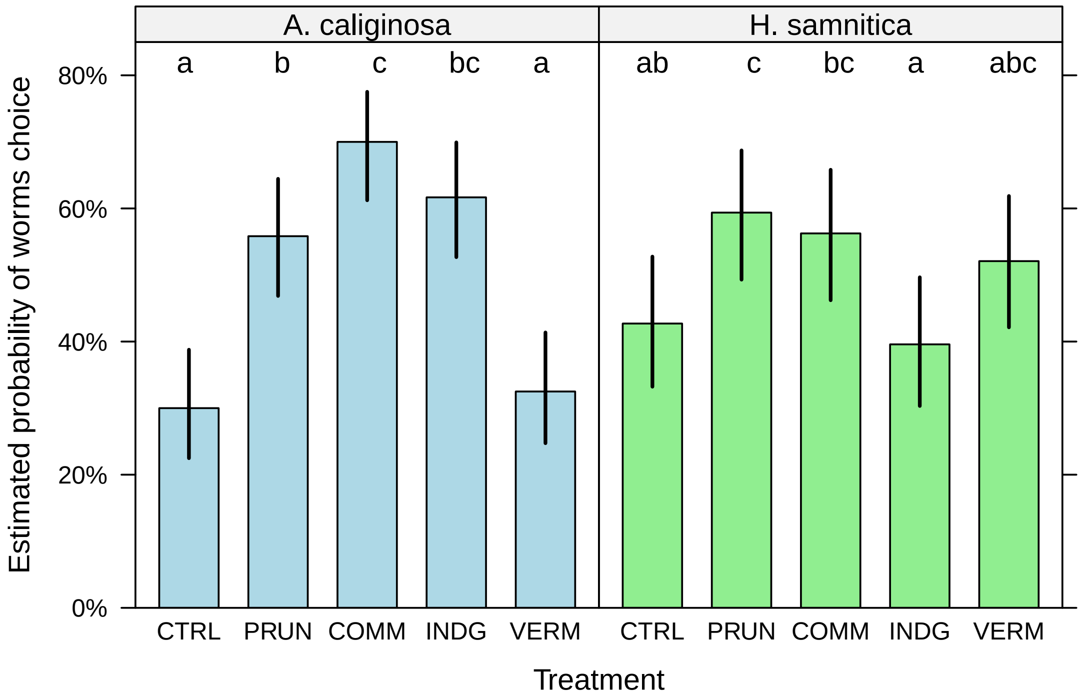


Figure 21: Estimated probability of soil side choice by *A. caliginosa* and *H. samnitica* across different organic amendment treatments in the two-choice preference test. Probabilities were estimated using a binomial generalized linear model fitted to the observed counts of individuals recovered on each side.Different letters indicate significant differences between treatments ($p < 0.05$; Tukey's post-hoc test).

## 6.7 Discussion

### 6.7.1 Characterization of the burrowing behaviour of *H. samnitica*

The first objective of this study was to characterize the bioturbation behavior of *H. samnitica* and to verify whether its subdivision into three weight classes could meaningfully explain differences in soil structural modifications and biological responses. Our findings confirm that individual size within this species plays a substantial role in determining its activity and ecological impact.

The absence of surface cast production beyond the first hours is in line with the endogeic nature of *H. samnitica*, whose activity remains confined to subsurface layers (Bouché 1977; Capowiez et al. 2024). Interestingly, casts were only observed in the presence of “medium” and “big” further indicating that these casts were made when burrowing the very first burrows.

Weight variation over the incubation period further supports this interpretation. While both “big” and “medium” individuals showed weight gain, “small” individuals exhibited a significantly lower weight increase, suggesting a reduced adaptability or lower feeding efficiency in the mesocosm conditions (Lowe and Butt 2002). This aligns with observations in other earthworm species, where juveniles often exhibit slower acclimation and reduced mobility in constrained environments (Grigoropoulou et al. 2009). It could also indicate that young *H. samnitica* would require soils with a higher organic matter content (or quality) to grow.

Structural analysis of the burrow system revealed a significant effect of body size on macropore diameter. The smaller class generated narrower burrows, with a marked difference compared to the “big” individuals, confirming that larger earthworms exert a greater mechanical influence on soil structure (Capowiez et al. 2015). Although total macroporosity and vertical distribution (barycenter) did not differ significantly among classes, the H2 layer macroporosity was significantly reduced in the “small” group. This suggests limited vertical exploration by smaller individuals, potentially due to lower burrowing capacity or energetic constraints as assumed for *L. terrestris* juveniles (Grigoropoulou et al. 2009).

Water infiltration patterns confirmed the functional relevance of these differences. The presence of “big” and “medium” earthworms preserved high infiltration rates, while the “small” class drastically reduced water movement, reflecting both their reduced macropore diameter and limited soil exploration. These results support previous findings indicating that earthworm-induced macropores, particularly deep, continuous ones, are critical for enhancing soil hydraulic conductivity (Pelíšek 2018; Shipitalo and Butt 1999).

Overall, this first characterization of *H. samnitica* under controlled conditions suggests that this species is purely endogeic and its impact on soil physical properties is size-dependent. Larger individuals appear better adapted to the experimental context and more effective in creating preferential flow paths and altering soil porosity.

### 6.7.2 Effects of organic amendments on *H. samnitica* bioturbation activity

The second objective of this study was to explore the effect of different organic amendments on *H. samnitica* behavior and soil modification capacity. Differences among treatments were most apparent in the upper soil layer (H1), where amendments were mixed with the soil, supporting the hypothesis that organic matter availability modulates burrowing behavior (Capowiez et al. 2021; Frazão et al. 2019). This is well known for endogeic species (Hugnes et al. 1996).

Compost-based treatments (COMM, PRUN, INDG) significantly enhanced macropore volume in the H1 layer (0-10 cm), likely reflecting an increased feeding or exploration activity in response to the presence of labile organic matter, as observed in other endogeic species which preferentially concentrate their burrowing activity in organic-rich layers (Capowiez et al. 2021; Le Couteulx et al. 2015). The vertical profile of macroporosity was also altered, with the COMM treatment inducing a marked shift of the barycenter toward the surface. This confirms that earthworm bioturbation was spatially influenced by amendment placement and suggests a positive response of *H. samnitica* to this type of amendment.

Interestingly, VERM treatment failed to induce significant changes in either macroporosity or barycenter position, mirroring what was observed for small individuals. This may be due to the more advanced decomposition stage of vermicompost and its reduced palatability, as previously suggested for other species (Ducasse et al. 2025, 2023).

Despite increased porosity in the upper layer, infiltration rates were reduced in compost-treated soils. This counterintuitive result may be explained by the formation of smaller, more tortuous macropores near the surface, perhaps less continuous and thus less insufficient to ensure rapid vertical flow. Alternatively, enhanced biological activity may have favored soil aggregate reorganization or partial pore clogging, especially in PRUN and COMM treatments.

### 6.7.3 Preference behavior and amendment attractiveness

The two-choice test results provide further insight into the behavioral responses of *H. samnitica* and *A. caliginosa* toward amended soils. Across the board, both species showed higher selection probabilities for PRUN, COMM, and INDG compared to CTRL, while VERM did not differ significantly from the CTRL.

Species-specific responses were particularly informative. *A. caliginosa* displayed a clear hierarchy of preferences, with COMM being the most attractive, consistent with its known ability to discriminate organic substrates based on OM quality and stage of decomposition (Le Couteulx et al. 2015). *A. caliginosa* is often classified as a specialist regarding its diet (Hsu et al. 2023). In contrast, *H. samnitica* showed less distinct preferences, suggesting a more conservative exploratory behavior. This weaker differentiation is coherent with its ecological strategy and limited mobility in short-duration assays.

The fact that vermicompost was not significantly preferred by either species reinforces the notion that substrate freshness and microbial composition, both more typical of composts, play a decisive role in short-term attractiveness (Ducasse et al. 2025). Furthermore, preference responses were consistent with the observed changes in macroporosity and infiltration, confirming that bioturbation intensity and behavioral choices are strongly interlinked and mediated by amendment characteristics.

### 6.7.4 Limitations and future directions

This study, while providing novel insights into the behaviour of *H. samnitica*, has some limitations. The short-term mesocosm design does not fully reproduce the complexity of field conditions, including seasonal variability and plant-soil interactions. In addition, only a limited number of amendment types and a single application rate were tested, whereas agricultural practices involve greater variability. Finally, the focus on individual earthworms did not capture potential density-dependent effects or interactions among different species. Future studies should therefore extend the duration and scale of observations, test a wider range of organic inputs and doses, and integrate multi-species approaches under realistic agronomic conditions to assess the long-term implications for soil structure, fertility, and crop performance.

## 6.8 Conclusions

*H. samnitica* exhibits size-dependent effects on soil structural properties, suggesting that individual biomass is a key determinant of its functional role in soil ecosystems. Larger individuals created wider and deeper macropores, and enhanced water infiltration that are critically important for soil functioning and hydrodynamics. Juveniles, as often for earthworms, live closer to the surface presumably in link with their less powerful muscles. These findings validate the subdivision into weight classes as ecologically meaningful, with smaller individuals showing limited adaptability, lower activity, and minimal influence on soil porosity and, in turn, on infiltration.

The application of organic amendments further modulated earthworm behavior and bioturbation. Compost-based treatments, particularly the compost inoculated with commercial composting accelerator (COMM), significantly increased macropore formation in the amended layer and altered its vertical distribution, confirming their capacity to stimulate biological activity. In contrast, vermicompost (VERM) had limited effects, consistent with its more advanced decomposition stage and reduced attractiveness. These structural changes, however, did not always translate into improved water infiltration, likely due to pore geometry or clogging effects.

Preference tests revealed that both *H. samnitica* and *A. caliginosa* were able to discriminate among substrates, favoring fresher, composted materials over the control and vermicompost. This supports the hypothesis that OM freshness and microbial composition are central to short-term substrate attractiveness and bioturbation induction.

Altogether, these results underscore the potential of specific amendments, particularly compost, to enhance soil structure and favor earthworm activity. They also highlight the need to consider species-specific responses and individual-level traits such as body size when assessing soil fauna reactions and effects. While the short-term mesocosm approach proved effective in differentiating amendment impacts, future studies should validate these findings under field conditions, where more complex interactions and environmental variability occur.

## 6.9 References

Bertrand M, Barot S, Blouin M, Whalen J, Oliveira T de, Roger-Estrade J (2015) Earthworm services for cropping systems. A review. Agron Sustain Dev 35:553–567. https://doi.org/10.1007/s13593-014-0269-7

Bouché M (1972) Lombriciens de france. Ecologie et systématique

Bouché MB (1977) Strategies lombriciennes. Ecological Bulletins 122–132

Canali S, Stopes C, Schmid O, Speiser B (2005) Current evaluation procedures for fertilizers and soil conditioners used in organic agriculture. Proceedings of a workshop held april 29–30, 2004 at emerson college, great britain. 100

Capowiez Y, Bottinelli N, Sammartino S, Michel E, Jouquet P (2015) Morphological and functional characterisation of the burrow systems of six earthworm species (lumbricidae). Biol Fertil Soils 51:869–877. https://doi.org/10.1007/s00374-015-1036-x

Capowiez Y, Gilbert F, Vallat A, Poggiale J-C, Bonzom J-M (2021) Depth distribution of soil organic matter and burrowing activity of earthworms—mesocosm study using x-ray tomography and luminophores. Biol Fertil Soils 57:337–346. https://doi.org/10.1007/s00374-020-01536-y

Capowiez Y, Marchán D, Decaëns T, Hedde M, Bottinelli N (2024) Let earthworms be functional - Definition of new functional groups based on their bioturbation behavior. Soil Biology and Biochemistry 188:109209. https://doi.org/10.1016/j.soilbio.2023.109209

Capowiez Y, Pierret A, Daniel O, Monestiez P, Kretzschmar A (1998) 3D skeleton reconstructions of natural earthworm burrow systems using CAT scan images of soil cores. Biol Fertil Soils 27:51–59. https://doi.org/10.1007/s003740050399

Ducasse V, Capowiez L, Peigne J, Capowiez Y (2025) Compost, digestate, and vermicompost from the recycling of urban biowaste have different impacts on earthworm behavior: A mesocosm study. Applied Soil Ecology 210:106095. https://doi.org/10.1016/j.apsoil.2025.106095

Ducasse V, Watteau F, Kowalewski I, Ravelojaona H, Capowiez Y, Peigné J (2023) The amending potential of vermicompost, compost and digestate from urban biowaste: Evaluation using biochemical, rock-eval® thermal analyses and transmission electronic microscopy. Bioresource Technology Reports 22:101405. https://doi.org/10.1016/j.biteb.2023.101405

FAO (2018) The 10 Elements of Agroecology. Guiding the Transition to Sustainable Food and Agricultural Systems.

Feller C, Blanchart E, Bernoux M, Lal R, Manlay R (2012) Soil fertility concepts over the past two centuries: The importance attributed to soil organic matter in developed and developing countries. Archives of Agronomy and Soil Science 58:S3–S21. https://doi.org/10.1080/03650340.2012.693598

Ferreira CSS, Seifollahi-Aghmiuni S, Destouni G, Ghajarnia N, Kalantari Z (2022) Soil degradation in the European Mediterranean region: Processes, status and consequences. Science of The Total Environment 805:150106. https://doi.org/10.1016/j.scitotenv.2021.150106

Frazão J, Goede RGM de, Capowiez Y, Pulleman MM (2019) Soil structure formation and organic matter distribution as affected by earthworm species interactions and crop residue placement. Geoderma 338:453–463. https://doi.org/10.1016/j.geoderma.2018.07.033

Frøseth RB, Bakken AK, Bleken MA, Riley H, Pommeresche R, Thorup-Kristensen K, Hansen S (2014) Effects of green manure herbage management and its digestate from biogas production on barley yield, n recovery, soil structure and earthworm populations. European Journal of Agronomy 52:90–102. https://doi.org/10.1016/j.eja.2013.10.006

Garrett RD, Ryschawy J, Bell LW, Cortner O, Ferreira J, Garik AVN, Gil JDB, Klerkx L, Moraine M, Peterson CA, Dos Reis JC, Valentim JF (2020) Drivers of decoupling and recoupling of crop and livestock systems at farm and territorial scales. E&S 25:art24. https://doi.org/10.5751/ES-11412-250124

Grigoropoulou N, Butt KR, Lowe CN (2009) Interactions of juvenile lumbricus terrestris with adults and their burrow systems in a two-dimensional microcosm. Pesq agropec bras 44:964–968. https://doi.org/10.1590/s0100-204x2009000800025

Hsu G-C, Szlavecz K, Csuzdi C, Bernard M, Chang C-H (2023) Ecological groups and isotopic niches of earthworms. Applied Soil Ecology 181:104655. https://doi.org/10.1016/j.apsoil.2022.104655

Hugnes MS, Bull CM, Doube BM (1996) Microcosm investigations into the influence of sheep manure on the behaviour of the geophagous earthworms aporrectodea trapezoides and microscolex dubuis. Biol Fert Soils 22:71–75. https://doi.org/10.1007/BF00384435

Kanwal AN (2021) A review on concerns about soil quality and innovative methods for improving soil health. ELSR 07:05–13. https://doi.org/10.31783/elsr.2021.720513

Le Couteulx A, Wolf C, Hallaire V, Pérès G (2015) Burrowing and casting activities of three endogeic earthworm species affected by organic matter location. Pedobiologia 58:97–103. https://doi.org/10.1016/j.pedobi.2015.04.004

Lenth RV (2017) Emmeans: Estimated marginal means, aka least-squares means

Lou XF, Nair J (2009) The impact of landfilling and composting on greenhouse gas emissions – A review. Bioresource Technology 100:3792–3798. https://doi.org/10.1016/j.biortech.2008.12.006

Lowe CN, Butt KR (2002) Influence of organic matter on earthworm production and behaviour:

A laboratory-based approach with applications for soil restoration. European Journal of Soil Biology 38:173–176. https://doi.org/10.1016/S1164-5563(02)01141-X

Manlay RJ, Feller C, Swift MJ (2007) Historical evolution of soil organic matter concepts and their relationships with the fertility and sustainability of cropping systems. Agriculture, Ecosystems & Environment 119:217–233. https://doi.org/10.1016/j.agee.2006.07.011

Marchán DF, Fernández R, De Sosa I, Sánchez N, Cosín DJD, Novo M (2018) Integrative systematic revision of a mediterranean earthworm family: Hormogastridae (annelida, oligochaeta). Invert Systematics 32:652. https://doi.org/10.1071/is17048

McCullagh P (2019) Generalized linear models, 2nd edn. Routledge, New York

Medina J, Monreal C, Barea JM, Arriagada C, Borie F, Cornejo P (2015) Crop residue stabilization and application to agricultural and degraded soils: A review. Waste Management 42:41–54. https://doi.org/10.1016/j.wasman.2015.04.002

Mombo S, Laplanche C, Besson P, Sammartino S, Schreck E, Dumat C, Capowiez Y (2018) Metal soil pollution differentially affects both the behaviour and exposure of a. Caliginosa and l. Terrestris: A mesocosm study. Biol Fertil Soils 54:319–328. https://doi.org/10.1007/s00374-017-1261-6

Omodeo P, Rota E (2008) Earthworm Diversity and Land Evolution in Three Mediterranean Districts. Proceedings of the California Academy of Sciences Fourth Series Volume 59:65–83

Pantani O-L, Ferretti L, Santoni M, Massenzio S, D'Acqui LP, Pacini GC (2022) Assessment of the impact of conventional and organic agroecosystems management options and conservation tillage on soil fertility at the montepaldi long term experiment, tuscany. European Journal of Agronomy 140:126575. https://doi.org/10.1016/j.eja.2022.126575

Pelíšek I (2018) Investigation of soil water infiltration at a scale of individual earthworm channels. Soil and Water Research 13:1–10. https://doi.org/10.17221/283/2014-SWR

R Core Team (2023) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Sarkar D (2001) Lattice: Trellis Graphics for R

Shipitalo MJ, Butt KR (1999) Occupancy and geometrical properties of lumbricus terrestris l. Burrows affecting infiltration. Pedobiologia 43:782–794. https://doi.org/10.1016/s0031-4056(24)00613-9

Vásquez Neyra JM, Cequea MM, Schmitt VGH (2025) Current practices and key challenges associated with the adoption of resilient, circular, and sustainable food supply chain for smallholder farmers to mitigate food loss. Front Sustain Food Syst 9: https://doi.org/10.3389/fsufs.2025.1484933

Wickham H (2023) Tidyverse: Easily install and load the tidyverse

Wickham H (2011) ggplot2. WIREs Computational Statistics 3:180–185. https://doi.org/10.1002/wics.147

Wickham H, François R, Henry L, Müller K (2021) Dplyr: A grammar of data manipulation

## 6.10 Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## 6.12 Author contributions

Francesco Serafini: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft.

Margherita Santoni: Methodology, Resources, Writing – review & editing, Supervision.

Ottorino-Luca Pantani: Resources, Investigation, Validation, Writing – review & editing.

Gaio Cesare Pacini: Supervision, Project administration, Writing – review & editing.

Yvan Capowiez: Conceptualization, Methodology, Supervision, Formal analysis, Validation, Writing – original draft , Writing – review & editing.

All authors have read and approved the final version of the manuscript.

## 6.13 Supplementary Materials

Table S19: Main characteristics of the soils sampled at different depths in the abandoned orchard and used to fill the soil core.

| Parameter | Top soil (0–15 cm) |
|---|---|
| Clay (%) | 19.7 |
| Silt (%) | 50.8 |
| Sand (%) | 29.5 |
| pH ($H_2O$) | 8.18 |
| CEC ($cmol^+ kg^{-1}$) | 8.48 |
| Organic matter (%) | 3.99 |
| C/N | 10.8 |
| N ($g\ kg^{-1}$) | 2.15 |
| P ($g\ kg^{-1}$) | 0.069 |
| K (Olsen) ($g\ kg^{-1}$) | 0.174 |

Table S20: Initial and final body weight (mean ± SD) of *Hormogaster samnitica* individuals and relative weight change (%) across organic amendment treatments.

| TRT | Initial weight (g) | Final weight (g) | Weight change (%) | n |
|---|---|---|---|---|
| CTRL | 4.75 (1.90) | 6.06 (2.96) | 25.0 (17.0) | 10 |
| PRUN | 4.67 (2.05) | 6.15 (4.07) | 26.4 (39.0) | 10 |
| COMM | 5.35 (3.66) | 8.18 (6.19) | 47.9 (17.0) | 10 |
| INDG | 4.89 (2.26) | 6.25 (2.75) | 29.6 (15.6) | 10 |
| VERM | 4.54 (2.74) | 6.40 (4.43) | 37.5 (20.3) | 10 |

Table S21: Results of ANOVA evaluating the effects of treatment (TRT), earthworm weight class (CLASS), and their interaction on earthworm performance and soil physical parameters. Significant p-values (< 0.05) are in bold.

| Variable | Factor | P value | F value |
|---|---|---|---|
| Weight change (%) | TRT | **0.034** | 2.875 |
| | CLASS | 0.108 | 2.343 |
| | TRT × CLASS | 0.528 | 0.806 |
| Infiltration rate (mm $min^{-1}$) | TRT | **3.3e-11** | 28.370 |
| | CLASS | 0.540 | 0.382 |
| | TRT × CLASS | 0.110 | 2.023 |
| Macropore diameter (mm) | TRT | **0.007** | 2.933 |
| | CLASS | **1.0e-09** | 2.578 |
| | TRT × CLASS | 0.870 | 0.309 |
| Macropore volume H1 layer ($cm^3$) | TRT | **1.1e-07** | 14.648 |
| | CLASS | 0.114 | 2.282 |
| | TRT × CLASS | 0.457 | 0.927 |
| Macropore volume H2 layer ($cm^3$) | TRT | 0.422 | 0.992 |
| | CLASS | **0.007** | 5.597 |
| | TRT × CLASS | 0.778 | 0.441 |
| Total macroporosity ($cm^3$) | TRT | **1.5e-06** | 11.712 |
| | CLASS | **5.0e-04** | 9.089 |
| | TRT × CLASS | 0.213 | 1.518 |
| Macroporosity barycenter (mm) | TRT | **0.016** | 3.435 |
| | CLASS | 0.107 | 2.356 |
| | TRT × CLASS | 0.326 | 1.195 |

Figure S17: *Hormogaster samnitica* individuals in the Montepaldi site

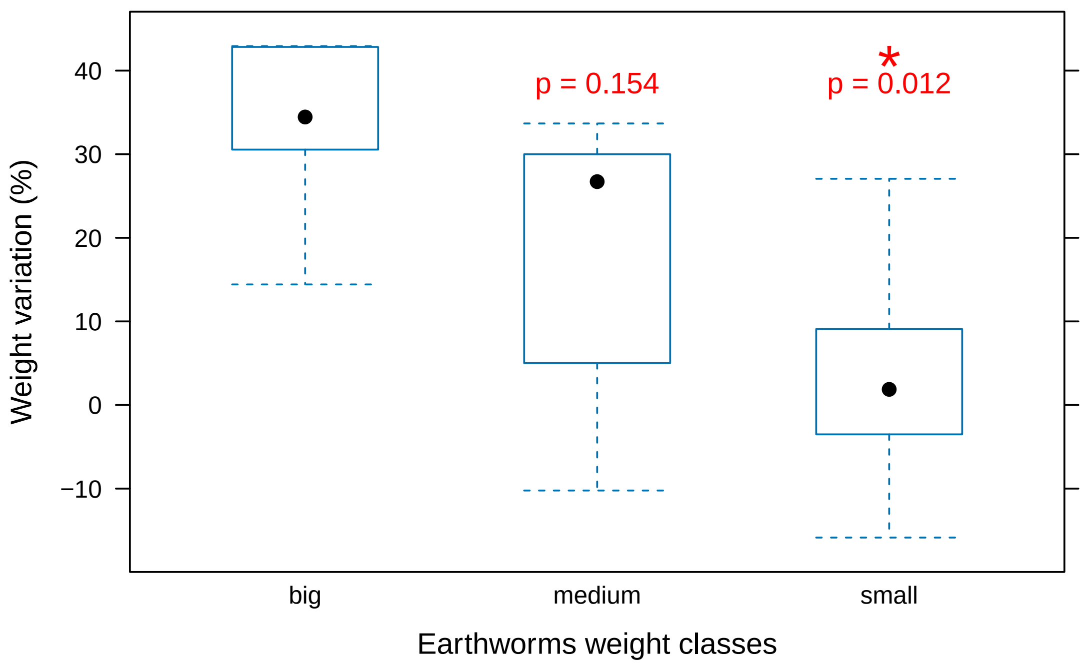


Figure S18: Weight variation (%) of *Hormogaster samnitica* individuals across different weight classes. Asterisks indicate statistically significant differences from the control treatment (CTRL): $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

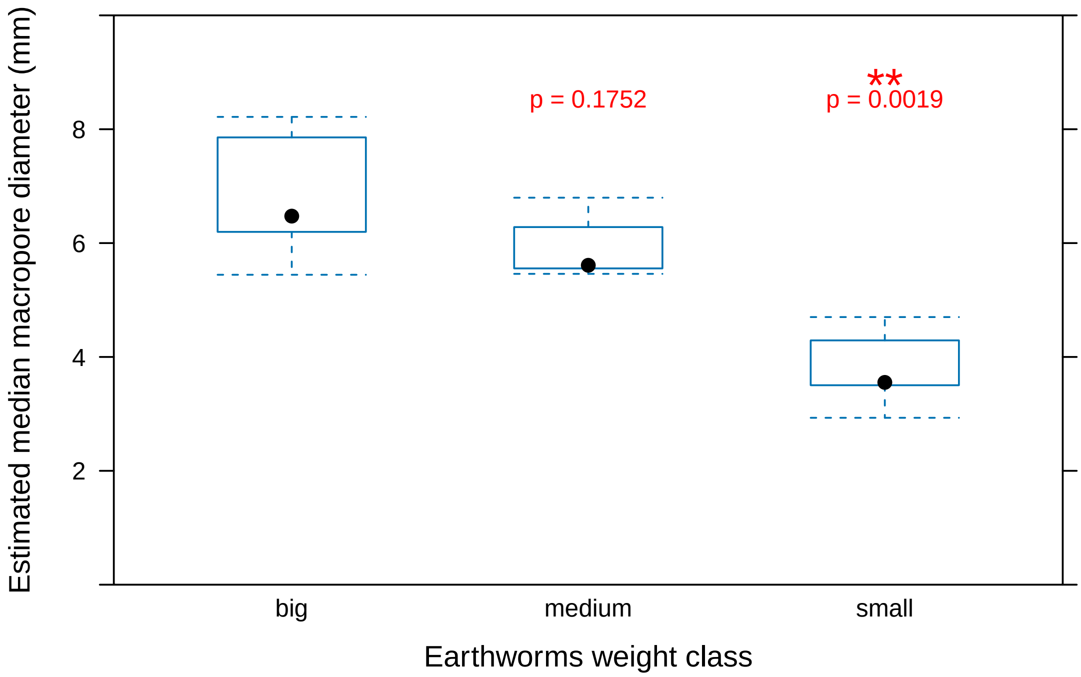


Figure S19: Estimated median macropore diameter of *H. samnitica* individuals across different weight classes. Asterisks indicate statistically significant differences from the control class (big): $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

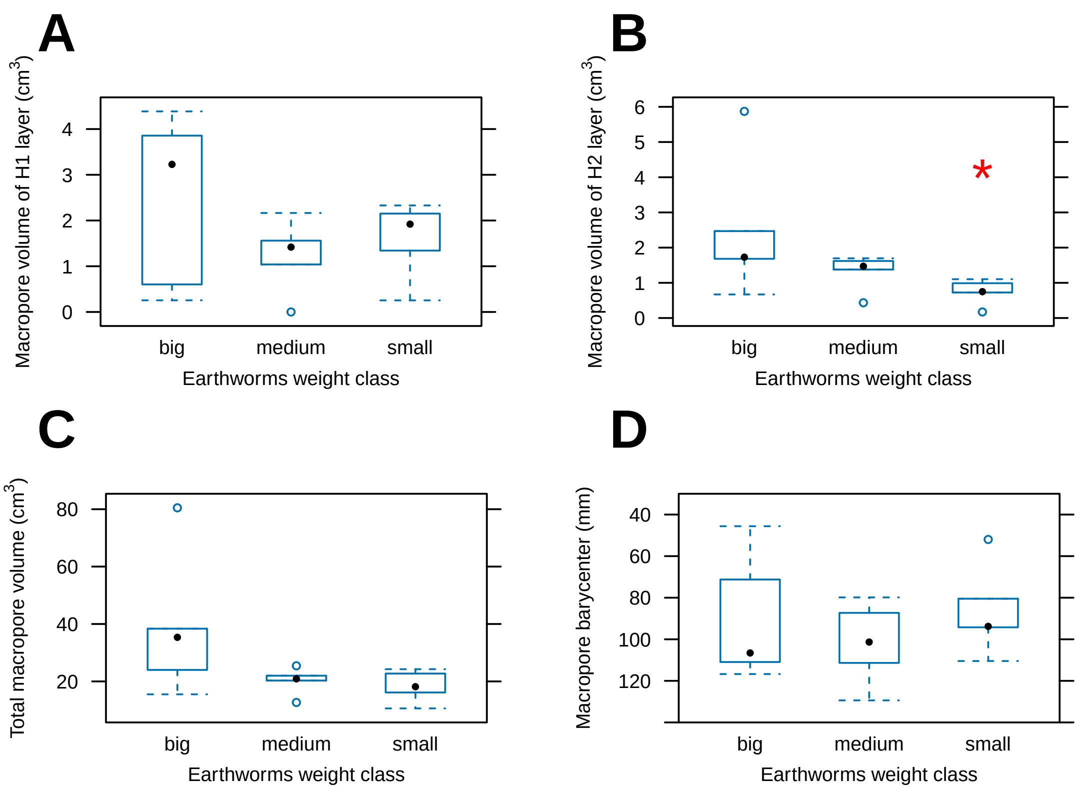


Figure S20: Effects of earthworm weight class on macropore characteristics. (A) Macropore volume in the H1 layer (0–10 cm), (B) Macropore volume in the H2 layer (10–26 cm), (C) Total macropore volume across the entire column (0–26 cm), (D) Vertical distribution of macroporosity (barycenter). Asterisks indicate statistical significance of pairwise comparisons versus "big" weight class: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

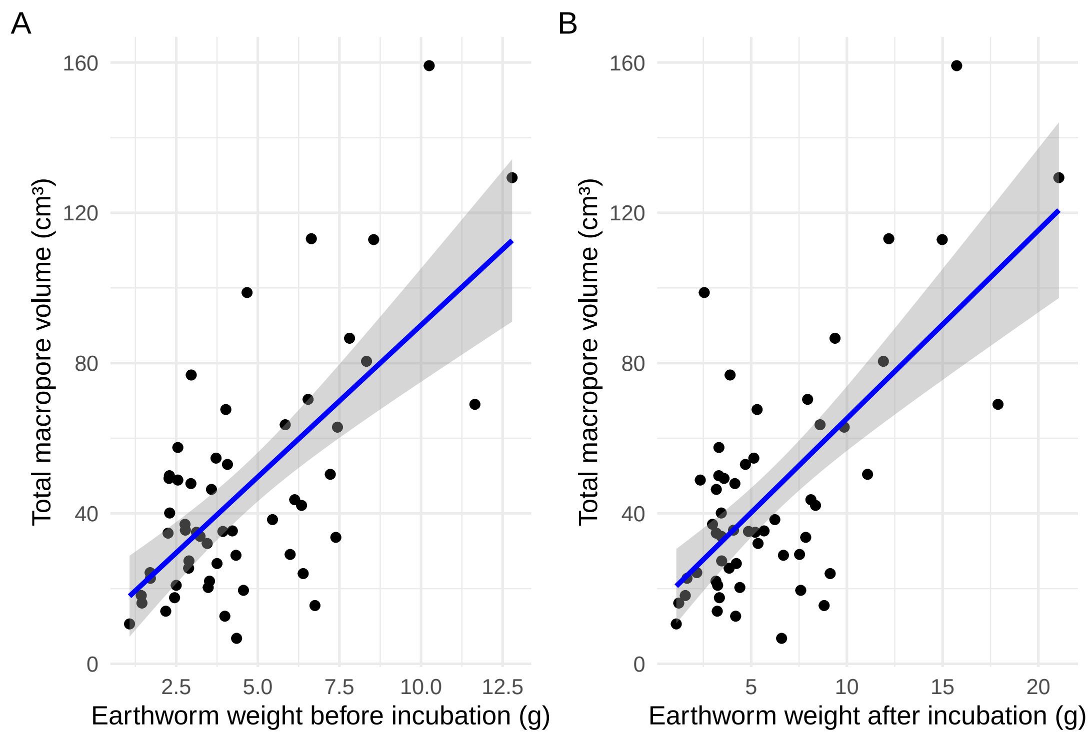


Figure S21: Relationship between earthworm weight (g) and total macropore volume ($cm^3$). (A) Linear regression between initial weight (before incubation) and total macropore volume; (B) linear regression between final weight (after incubation) and total macropore volume.

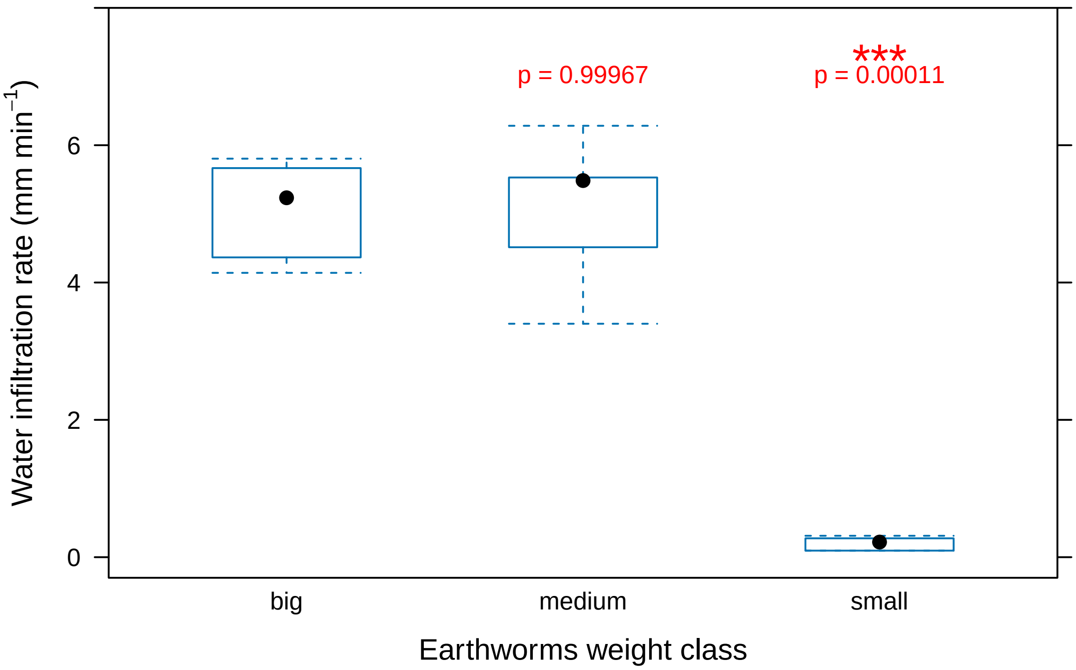


Figure S22: Water infiltration rate ($\mathrm{mm\,min^{-1}}$) measured in soil columns under different earthworm weight class. Asterisks denote statistically significant differences from the "big" weight class based on pairwise comparisons: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

# 7 Plant Response In Soil Amended With Vermicompost and Composted Vine Pruning: A Pot Experiment

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## 7.1 Abstract

The decline of organic matter and recurrent water scarcity in Mediterranean soils call for sustainable strategies that recycle local residues while improving agroecosystems functionality. This study evaluates the short-term effects of four organic amendments on soil properties and lettuce performance under controlled pot conditions. Vermicompost from spent coffee grounds (*VERM*), composted vine prunings (*PRUN*), pruning-derived compost with a commercial accelerator (*COMM*) and pruning-derived compost inoculated with Indigenous Microorganisms (*INDG*) were compared with an unamended control (*CTRL*). All treatments were applied at four rates (2, 8, 16, 32 t $ha^{-1}$). Two consecutive crop phases were planned on the same amended substrate: emmer wheat (*Triticum dicoccum*) was sown first (Feb - Jul 2024) but did not reach maturity under pot conditions; the experiment then continued with a second crop phase using lettuce (*Lactuca sativa* var. *capitata* 'Iceberg', Dec 2024 - Jul 2025) after re-applying the same amendment–dose combinations. Vermicompost increased substrate volumetric water content, with effects already evident at the lowest dose. Electrical conductivity increased slightly under *VERM* at high doses but remained within safe thresholds. Lettuce grown in *VERM* amended substrate displayed the highest shoot biomass and leaf number (+4.5 leaves compared to *CTRL*) and a greater shoot-to-root ratio, indicating preferential allocation to aboveground tissues. Physiological parameters (SPAD, colorimetric traits, Fv/Fm) did not differ significantly among treatments; Fv/Fm values remained above 0.83, confirming the absence of photoinhibition. Overall, VERM was proved particularly effective in enhancing water retention and crop growth under Mediterranean conditions, even at moderate application rates, while pruning-derived composts showed less evident agronomic benefits.

## 7.2 Keywords

Agroecology, Circularity, Organic Amendments, Plant Physiology, Soil Water Content

## 7.3 Introduction

Food and forage production has been based on for centuries on the management of manures, crop residues, and other by-products, i.e. organic matter (OM) whose mass is essentially carbon (Manlay et al. 2007). In a certain sense, it can be said that the goal of nourishing plants was achieved by tuning the carbon cycle in and out of the soil.

The rise of modern agriculture disrupted this cycle. Many aspects of current farming systems, such as monocropping, intensive livestock, the separation of crops from livestock systems, and reliance on synthetic fertilizers have greatly reduced the reintegration of OM into soils (Feller et al. 2012). As a result, large amounts of organic residues are today treated as waste, burned or left unmanaged, thus contributing to greenhouse gas emissions and atmospheric pollution (Lou and Nair 2009).

This transformation in the management of Organic Wastes (OW) has coincided with a marked decline in SOM across many regions, particularly in the Mediterranean basin (Ferreira et al. 2022; Montanarella et al. 2016). Soil OM is central to its fertility: it stabilizes aggregates, retains water and nutrients, supports microbial activity, and buffers climate variability (Lal 2004). Its depletion therefore reduces soil resilience to climatic variability and to management pressures, increases erosion risks, and mines long-term productivity. Addressing SOM decline and OW mismanagement thus represents a double challenge for Mediterranean agriculture.

Globally, crop residues exceed 70 Tg annually, with rice and wheat contributing the largest share (45 Tg) (Medina et al. 2015). In viticultural areas, widely distributed across the Mediterranean, vineyard prunings represent several tonnes per hectare each year, yet they are still frequently burned or left to decay unmanaged. Such disposal practices reflect a mismatch between resource availability and soil needs, highlighting the urgency of developing strategies to bring OW back into agricultural systems.

The principles of agroecology and circular economy provide a coherent framework for such strategies. Agroecology emphasizes nutrient recycling, crop diversification, and reduced reliance on external inputs (Altieri and Nicholls 2017), while circular economy promotes the transformation of waste into valuable inputs, reducing disposal costs and enhancing resource efficiency (FAO 2018). Both agroecology and circular economy point to composting and vermicomposting as valid options to restore SOM levels while reducing the environmental footprint of farming.

Composting processes stabilize OW and generate an amendment which improves soil fertility and structure. Compost applications enhance aggregation of soil particles, increase CEC, and build organic carbon stocks (Diacono and Montemurro 2010). Notably, compost improves soil water-holding capacity (Bernal et al. 2009), a function of increasing significance under climate change scenarios characterized by droughts and irregular rainfall (Iglesias and Garrote 2015; Smith et al. 2020).

Vermicomposting offers a complementary pathway to simple composting, where OW are processed by earthworms to produce a finer product characterized by low bulk density, good structure, and high moisture-holding capacity (Dominguez and Edwards 2010). When compared with conventional composts vermicompost has been associated with greater improvements in soil porosity, soil water retention, and plant growth (Lim et al. 2012). These properties make vermicompost an increasingly relevant option in Mediterranean systems.

The agronomic value of both compost and vermicompost extends beyond soil fertility. They have been shown to enhance yields and reduce dependence on chemical fertilizers (Bhardwaj et al. 2014; Lazcano and Dominguez 2011). However, their effects vary depending on feedstock, stabilization degree, and processing methods (Bernal et al. 2009). Pruning residues, for instance, are lignin-rich and may yield composts of lower short-term agronomic value, while vermicompost can act as a biostimulant due to its finer texture and enriched microbial composition.

In Mediterranean environments, water scarcity highlights the importance of OW recycling. Water availability often has a stronger influence than nutrients on biomass allocation in leafy vegetables, where shoot growth is particularly sensitive to soil moisture (Poorter et al. 2012; Rouphael et al. 2012). Enhancing soil water retention through organic amendments may therefore serve as a key strategy to sustain productivity under climate variability. At the same time, composts can increase soil salinity, and excessive values may be detrimental for crops, underscoring the need to balance benefits against potential risks.

The present pot experiment evaluated the short-term effects of four organic amendments on selected soil properties and plants physiological and growth traits. Vermicompost derived from spent coffee grounds (*VERM*) was tested alongside three composts obtained from vine pruning residues (*PRUN*, *COMM*, *INDG*), each prepared with different composting strategies and inoculation. An unamended soil served as control (*CTRL*).

The experiment consisted of two sequential crop phases conducted on the same pots/substrate. In the first phase, emmer wheat (*Triticum dicoccum*) was grown from sowing to the end of the growing period (Feb - Jul 2024) but failed to reach maturity. For the second phase, the substrate from each pot was recovered, homogenized, and re-amended with the same treatment and dose previously assigned, after which lettuce (*Lactuca sativa* var. *capitata* 'Iceberg') was transplanted (Dec 2024 - Jul 2025). Therefore, "two crop phases" indicates two consecutive crops within the same pot experiment, not independent experiments. Specifically, the study aimed to: (i) examine the impact of vermicompost and composts from vine prunings on soil water retention capacity and salinity; (ii) evaluate whether improvements in soil conditions translated into enhanced plant growth and biomass allocation; and (iii) determine whether physiological indicators such as SPAD index, chlorophyll fluorescence, and leaf colorimetry revealed differences in nitrogen status or photosynthetic performance.

By integrating substrate (SP), plant (PP) and physiological (PhP) responses, this experiment contributes to assess residue-derived amendments as practical tools for sustainable soil management in Mediterranean systems, highlighting the potential of circular economy strategies to recycle OW into resources that improve substrate fertility and crop resilience.

## 7.4 Material and Methods

### 7.4.1 Experimental design

The experiment followed a fully factorial design with two fixed factors: (i) organic amendment type (five levels: *CTRL*, *PRUN*, *COMM*, *INDG*, *VERM*) and (ii) application rate (four levels: 2, 8, 16, and 32 t $ha^{-1}$, dry matter basis). Each amendment × rate combination was replicated eight times (n = 8), resulting in a total of 160 pots (5 amendments × 4 doses × 8 replicates). Pots were arranged in eight completely randomized blocks to minimize positional effects. Within each block, all treatment–dose combinations were randomly assigned to individual pots. A schematic representation of the experimental layout and pot allocation is provided in Fig. S23.

### 7.4.2 Site and Soil Sampling

The soil was collected from the Montepaldi Long Term Experiment (MoLTE, located in Montepaldi, San Casciano Val di Pesa, Italy long. 11°09'08'' E, lat. 43°40'16'' N, 90 m a.s.l.) in January 2024. The soil is classified as Fluventic Xerochrepts and has a texture between silty clay loam and clay loam. The soil was passed through a 2 cm mesh to remove large debris. The soil' main physico-chemical characteristics are reported in Table S22.

### 7.4.3 Organic amendment treatments

Four organic amendments (*VERM*, *PRUN*, *COMM* and *INDG*) plus the unamended control (*CTRL*) were tested. VERM consisted of vermicompost derived from spent coffee grounds and supplied by the company Funghi Espresso Società Agricola s.r.l.. Vine pruning residues (about 15 q) were collected at Castello del Trebbio (Pontassieve, Florence, Italy)in April 2023 and 2024 and were blended in 1:1, w/w ratio with alfalfa to increase the C/N ratio. This mixture formed the source material for *PRUN*, *COMM* and *INDG* which was shredded and then composted at the Montepaldi Long Term Experiment (MoLTE) site. *PRUN* consisted of composted vine prunings without any inoculant. *COMM* was produced by adding a commercial composting accelerator (600 g $m^{-3}$) during pile establishment. *INDG* was prepared by inoculating the source material with Indigenous Microorganisms ($IMO_2$) following the Korean Natural Farming protocol (Keliikuli et al. 2019). The $IMO_2$ inoculum was obtained in two stages ($IMO_1$, collection and $IMO_2$, propagation) and applied at 500 g diluted in 10 L of water per pile. Unlike *VERM* the composts from vine residues originated from a different substrate and showed distinct physical and chemical properties.

Temperature dataloggers were buried into the composting piles, which were turned and moistened regularly to maintain aerobic conditions and enhance microbial activity. The composting process was repeated separately for each crop phase (2024 and 2025), ensuring that amendments were freshly produced and applied in both years. The composting stage was performed in May and lasted about 24 weeks.

The main physico-chemical properties of the four amendments are reported in Table 5.

Table 5: Averages (± standard deviation) of the physico-chemical qualities of the organic amendments, determined according to internationally recognized standard procedures (ISO, TMECC) (n = 3).

| Treatment | Density (kg $L^{-1}$) | Moisture (%) | pH | EC (mS $cm^{-1}$) | C (%) | N (%) | C/N |
|---|---|---|---|---|---|---|---|
| PRUN | 0.13 (0.01) | 13.2 (0.25) | 6.9 (0.05) | 0.90 (0.36) | 43.9 (0.67) | 0.80 (0.03) | 57.4 (3.39) |
| COMM | 0.13 (0.01) | 13.5 (0.40) | 6.9 (0.02) | 0.80 (0.02) | 44.5 (0.24) | 0.80 (0.02) | 58.0 (1.61) |
| INDG | 0.15 (0.01) | 12.8 (0.06) | 7.0 (0.01) | 0.70 (0.02) | 43.6 (1.34) | 0.70 (0.05) | 62.6 (3.03) |
| VERM | 0.49 (0.01) | 67.9 (0.78) | 5.8 (0.05) | 0.90 (0.06) | 42.8 (1.53) | 5.00 (0.19) | 8.5 (0.03) |

### 7.4.4 Experimental setup

**7.4.4.1 Substrate Formation and Potting** Each amendment was applied at four rates, corresponding to field application doses of 2, 8, 16, and 32 t $ha^{-1}$. The amendments were thoroughly incorporated into the soil using a portable concrete mixer to ensure uniform distribution, and pots were then filled to contain approximately 4 kg of substrate (soil + amendment) each.

The pot experiment was conducted at the Department of Agricultural, Food, Environmental and Forestry Sciences (DAGRI), University of Florence.

The experimental design was maintained across sequential crop phases. For the second cycle, pot replicates ($n^{th}$ amendment and $m^{th}$ dose) were emptied into the concrete mixer, and the substrate added with identical amendment and dose previously used.

**7.4.4.2 Seedling Establishment** The first cycle was conducted with emmer wheat (*Triticum dicoccum*). Sowing was performed on 9 February 2024, with 10 seeds per pot to evaluate germination rate. Seedling emergence was monitored regularly, and germinated seedswere recorded on March 4th, 2024. Germination rates did not fall below 70% in any pot, and no significant interaction with amendment type or application dose was found statistically significant ($p > 0,05$) (Figure S25). Plants were then thinned to 3 per pot to simulate field density. Despite successful emergence and early vegetative development, plants did not reach reproductive maturity by July 2024. This failure was most likely due to pot size constraints limiting root expansion and nutrient acquisition in a cereal species with high spatial requirements. As a consequence, no meaningful quantitative measurements of plant growth and yield could be collected for this crop phase.

After emmer wheat failing to reach maturity,the experimental setup was repeated in the second cycle using lettuce (*Lactuca sativa var. capitata* 'Iceberg'). One lettuce seedling was transplanted into each pot on 2 December 2024, after amendments had been reapplied on 21 November 2024.

### 7.4.5 Measurements

**7.4.5.1 Substrate Parameters (SP)** Substrate conditions were monitored to evaluate amendment effects. The substrate was saturated twice during the first crop phase (25 June and 10 July 2024) and once in the second one (14 July 2025), and volumetric water content (VWC) and pore water electrical conductivity (ECp) were measured (HH2 Moisture Meter, Delta-T Devices, Cambridge, UK; Fig. S24) 7 and 14 days after each saturation event.

To improve monitoring accuracy and temporal resolution, during the second cycle, sensors connected to a datalogger were installed in selected pots corresponding to the *CTRL* treatment and to the highest *VERM* dose (32 t $ha^{-1}$, VERM32). Sensors (TEROS 12, METER Group, Pullman, WA, USA) were connected to the datalogger (ZL6, METER Group, Pullman, WA, USA) to record VWC, ECp, and temperature every 30 minutes,

The choice of monitoring only *CTRL* and VERM32 pots was guided by the data collectedin the first crop phase, which indicated positive effects of *VERM* at higher doses, and by technical limitations of the datalogger, which allows a maximum of six sensor connections. To ensure three replicates per treatment, three sensors were installed in three *CTRL* pots and three in three VERM32 pots. The specific pots used were randomly chosen to avoid positional bias.

**7.4.5.2 Plant and Physiological Parameters (PP and PhP)** At lettuce harvest, both non-destructive and destructive measurements were performed on plants. Destructive analyses included leaf number, shoot fresh and dry biomass, root dry biomass, and shoot-to-root ratio. Non-destructive measurements included PhP such as leaf chlorophyll content, chlorophyll fluorescence, and leaf colorimetry.

Leaf chlorophyll content was determined with a SPAD meter (SPAD-502Plus, Konica Minolta, Osaka, Japan; Fig. S24), while photosynthetic efficiency was assessed using a Handy PEA+ fluorimeter (Hansatech Instruments, UK; Fig. S24). In particular, the maximum quantum efficiency of photosystem II (Fv/Fm) was analyzed, which is widely recognized as a reliable indicator of photosynthetic performance and plant stress status (Maxwell and Johnson 2000). Dark-adapted Fv/Fm values close to 0.83 are generally considered optimal for most plant species, whereas lower values reflect the occurrence of stress and photoinhibition (Björkman and Demmig 1987; Johnson et al. 1993).

Leaf color was assessed with a colorimeter (CM-700D, Konica Minolta, Osaka, Japan), which provided values in the CIE Lab* system: L* (lightness, 0 = black, 100 = white), a* (green to red axis, negative = green, positive = red) and b* (blue to yellow axis, negative = blue, positive = yellow).

From these primary parameters, two additionally indices were derived:

- Chroma (C*), an indicator of colour intensity, calculated as:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

- Hue angle (h°), describing the overall shade of color, calculated as:

$$h^\circ = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

These indices have been widely applied in horticultural studies to better characterize plant colouration and pigment development (Najafzadeh et al. 2014).

The colorimeter also provided reflectance measurements under two different conditions: SCI (Specular Component Included) and SCE (Specular Component Excluded). For consistency with previous studies (Najafzadeh et al. 2014) and the present experimental design, only one of the two modes (SCE) was selected for analysis.

**7.4.5.3 Statistical Analysis** All statistical analyses were performed with R version 4.5.0 (R Core Team 2023). Data preprocessing and aggregation were carried out with dplyr and tidyr packages (Wickham 2016), while graphical outputs were generated with lattice (Sarkar 2001).

Substrate parameters (SP: volumetric water content, VWC; pore water electrical conductivity, ECp) and most plant parameters (PP: shoot fresh and dry biomass, root biomass, shoot-to-root ratio, leaf moisture, SPAD index, and colorimetric traits) were fitted by linear models including treatment, dose, and their interaction as explanatory factors. Leaf number, being a count variable, was fitted with a generalized linear model assuming a Poisson distribution and a log-link function. For all data classes, alternative marginal models were compared by likelihood ratio tests in order to identify the simplest one capable of explaining the observed variability.

In addition to the full models, planned contrasts restricted to the pruning derived composts (*PRUN*, *COMM*, *INDG*) to isolate the effect of inoculation were performed. In this case the reference treatment was the uninoculated compost (*PRUN*). Because these targeted contrasts detected no significant differences relative to *PRUN* across any indicator, detailed results are not reported.

Sensor-based time series (VWC, ECp) were compared between treatments using paired t-tests applied directly to the two temporal vectors.

Statistical significance was reported where $p < 0.05$.

## 7.5 Results

### 7.5.1 First crop phase (2024, *Triticum dicoccum*)

**7.5.1.1 Substrate Physicochemical Parameters** During the first crop phase, substrate VWC was significantly influenced by the type and dose of organic amendments (Table S23, Figures 22 and 23). INDG consistently reduced VWC compared to the CTRL, especially at 16 t ha$^{-1}$, while VERM increased water content, particularly at 16 and 32 t ha$^{-1}$. COMM reduced VWC only at 32 t ha$^{-1}$, whereas PRUN did not differ significantly from the CTRL.

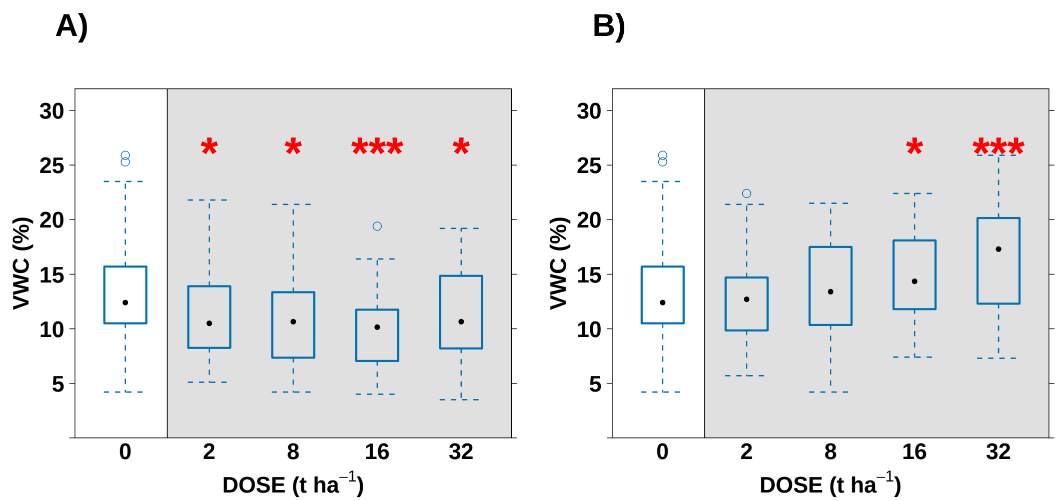


Figure 22: Volumetric water content (VWC) of the substrate measured under 0, 2, 8, 16 and 32 t ha$^{-1}$ application rates of: A) INDG and B) VERM. Asterisks indicate statistical significance of pairwise comparisons against dose 0 (CTRL): $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

These patterns are illustrated in Figures 22 and 23: INDG caused significant reductions (p < 0.05) in VWC compared with CTRL across all doses while VERM showed a significant positive dose-dependent effect.

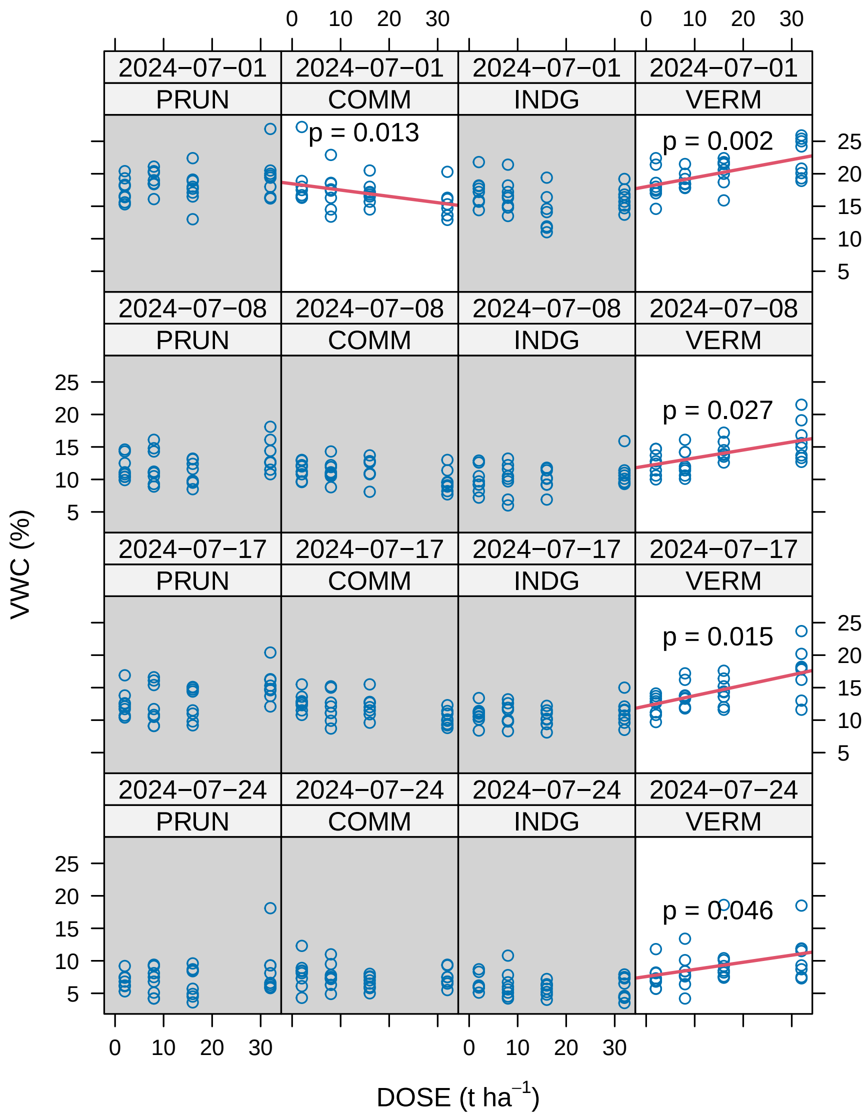


Figure 23: Relationships between volumetric water content (VWC, %) of substrate and application rates (t ha$^{-1}$) for each treatment (PRUN, COMM, INDG, and VERM) across four sampling dates (2024-07-01, 2024-07-08, 2027-07-17 and 2024-07-24) during the first crop phase (2024, Triticum dicoccum). p-values indicate the statistical significance of the correlation. Gray panels indicate non-significant relationships at $p > 0.05$.

Table 6: Summary for the linear model fitted to the differences in substrate volumetric water content (VWC, %) between 7 and 14 days after saturation during the first crop phase (2024, *Triticum dicoccum*).

| Treatment | Estimate | p-value |
|---|---|---|
| CTRL | 5.67 | $< 10^{-3}$*** |
| PRUN | 0.51 | 0.411 |
| COMM | -0.46 | 0.453 |
| INDG | -0.44 | 0.481 |
| VERM | 0.07 | 0.908 |

To assess whether amendments might influence the rate of water loss from the substrate, VWC between 7 and 14 days after saturation were compared (Table 6). No significant effects due to amendments were detected, suggesting that the observed differences were due to the initial water retention capacity rather than to drying dynamics.

During the first crop phase, ECp displayed limited and treatment-specific dose responsesthan substrate VWC (Table S25). Examining the treatment-dose relationship, a significant positive slope emerged only for VERM on 17 July 2024 ($p = 0.028$), whereas the remaining VERM dates and all PRUN, COMM, and INDG panels were non-significant or showed sporadic differences without a coherent trend across doses (Fig. 24).

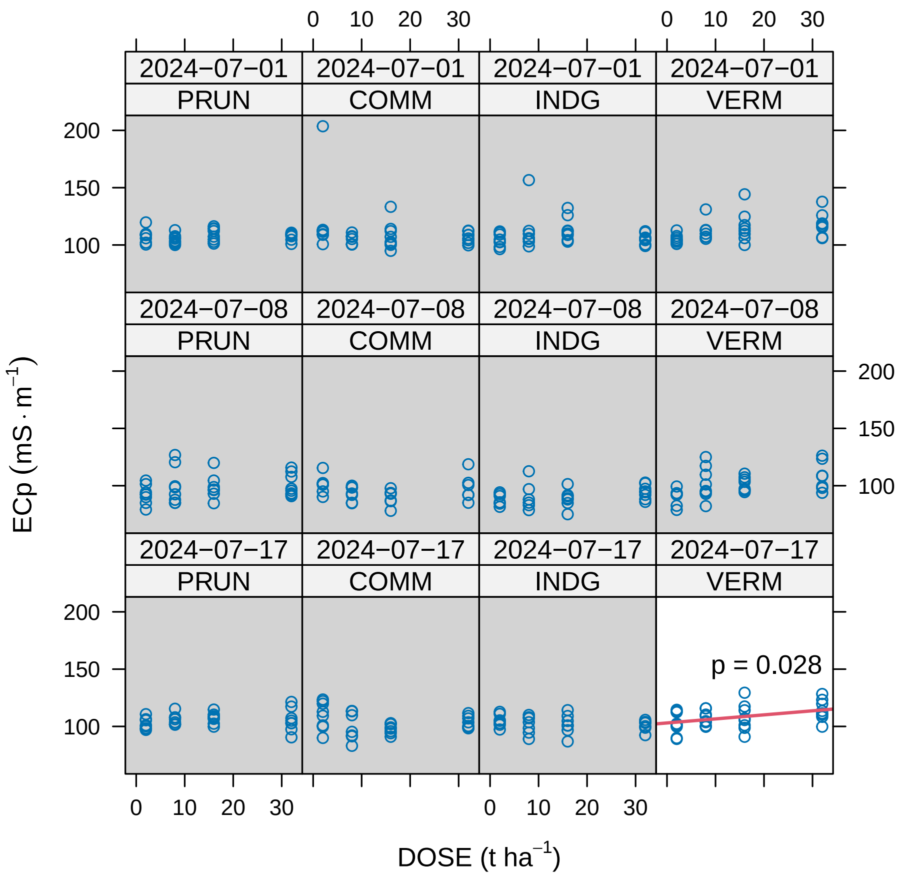


Figure 24: Relationships between electrical conductivity (ECp, mS $m^{-1}$) of substrate and application rates (t $ha^{-1}$) for each treatment (PRUN, COMM, INDG, and VERM) across four sampling dates (2024-07-01, 2024-07-08 and 2027-07-17) during the first crop phase (2024, Triticum dicoccum). p-values indicate the statistical significance of the correlation. Gray panels indicate non-significant relationships at $p > 0.05$.

### 7.5.2 Second crop phase (2025, *Lactuca sativa*)

**7.5.2.1 Substrate Physicochemical Parameters** During the second crop phase, substrate VWC was significantly influenced by the amendment (Table S23). In particular, *VERM* consistently increased it compared to the *CTRL*, with significant differences already detectable at 2 t ha$^{-1}$ and persisting across all higher application rates. In 2024 (*Triticum dicoccum*), linear models detected significant increases at doses higher than 16 t ha$^{-1}$ only, whereas in 2025 *VERM* enhanced substrate water retention even at the lowest application rate, indicating an additive effect during the second crop phase (Fig. 25).

Pruning derived composts showed only sporadic increases (*PRUN* at 2 t ha$^{-1}$, *COMM* at 16 and *INDG* at 32 t ha$^{-1}$).

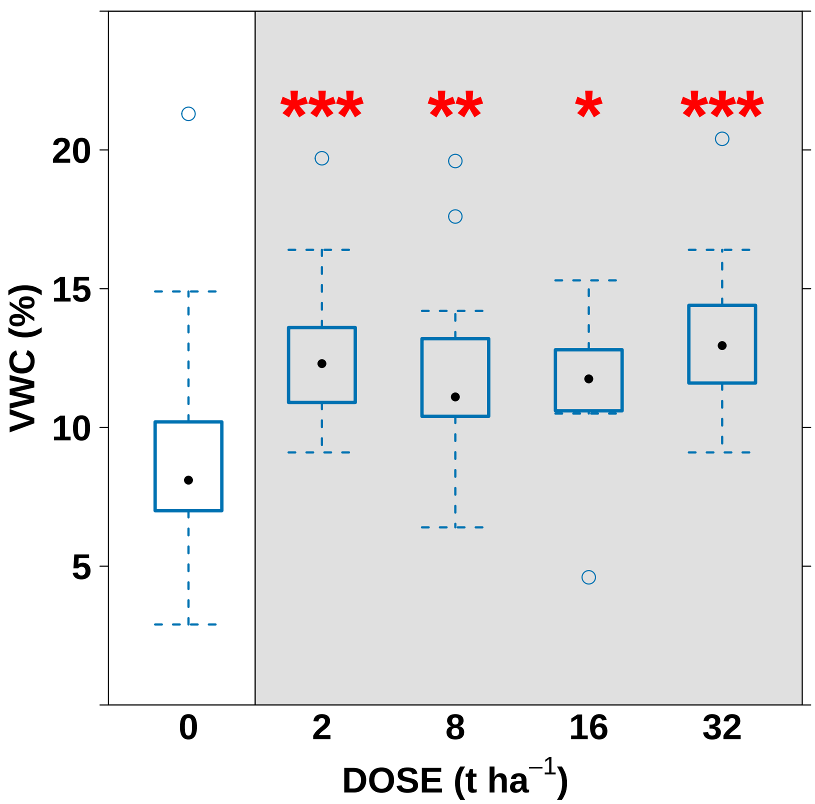


Figure 25: Volumetric water content (VWC) of the substrate measured under different application rates of VERM (0, 2, 8, 16 and 32 t ha$^{-1}$) during the second crop phase (Lactuca sativa, 2025). Asterisks indicate statistical significance of pairwise comparisons versus dose 0 (CTRL): $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

Also during the second crop phase substrate ECp showed less consistent effects of treatments compared to VWC (Table S24). Across both sampling dates, most amendments showed values close to the *CTRL*, with no clear dose-dependent responses.

Continuous monitoring of substrate VWC with datalogger provided additional insights into the effects of treatments (Fig. 26). Throughout the entire period, pots amended with VERM at the highest dose (VERM32) continuosly maintained higher VWC values than the *CTRL*. This is evident in the ΔVWC panel (VERM32 - CTRL), which remained predominantly positive over time (Fig. 26B).

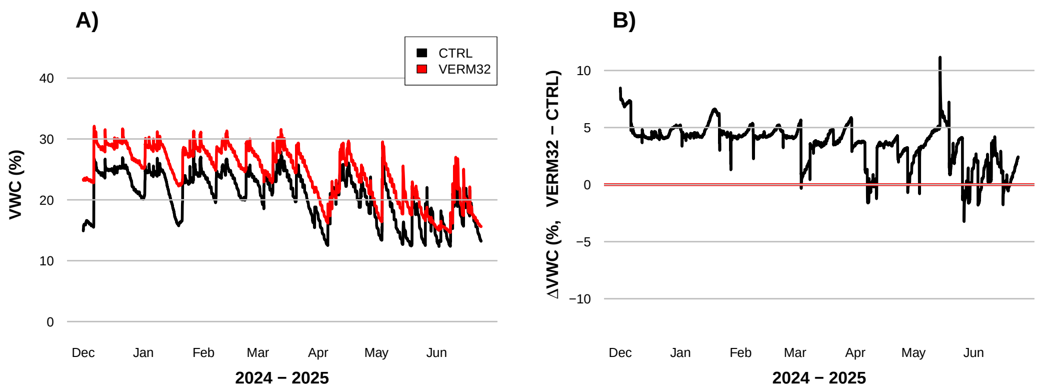


Figure 26: Substrate water dynamics during the second crop phase (2025, Lactuca sativa). A) substrate volumetric water content (VWC, %) and B) Difference in VWC (ΔVWC, %, VERM32 - CTRL). Measurements were collected at 30-minute intervals.

Paired t-tests performed on daily mean values substantiate these differences (Table S26). On average, VERM32 increased VWC by 3.6% relative to CTRL ($p < 10^{-3}$).

**7.5.2.2 Plant Growth and Biomass Allocation** Shoot dry weight was significantly affected by the amendment but not by the application rate (Fig. 27A). Lettuce plants grown in substrates amended with *VERM* exhibited the highest values, with an increase of 2.45 g on average compared to *CTRL* ($p < 0.01$).

Root dry weight also varied among treatments (Fig. 27B). The strongest positive effects were observed for *COMM* (+0.46 g, $p < 0.01$) and *INDG* (+0.33 g, $p < 0.01$), while *PRUN* and *VERM* did not significantly differ from *CTRL* ($p > 0.05$).

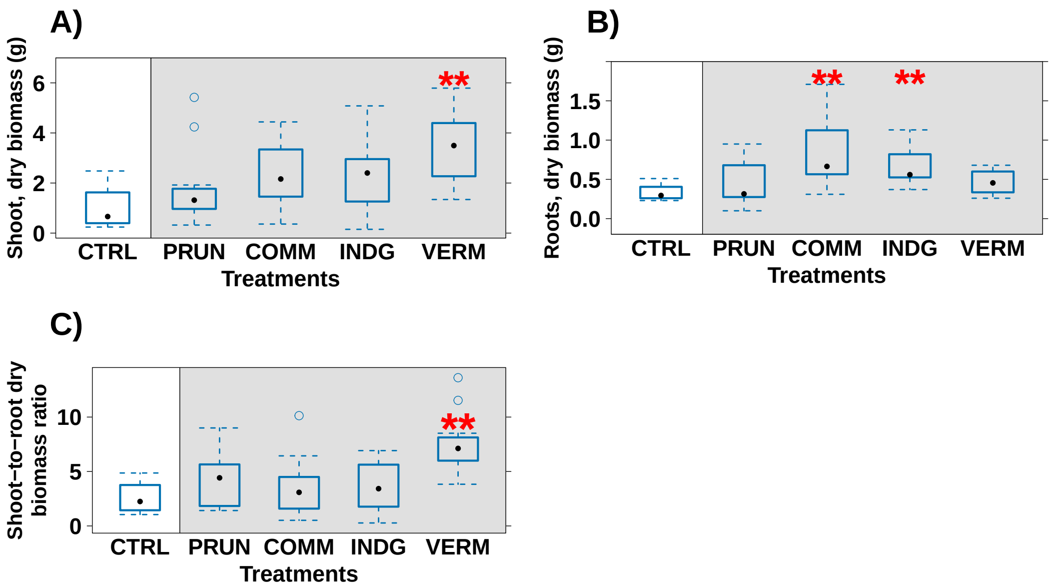


Figure 27: Effects of organic amendments on lettuce biomass allocation. (A) Shoot dry biomass, (B) Root dry biomass, and (C) Shoot-to-root dry biomass ratio under different treatments. Asterisks indicate statistical significance of pairwise comparisons versus CTRL: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

As a consequence, the shoot-to-root ratio was markedly affected by amendments (Fig. 27C). Vermicompost-amended plants displayed the highest ratios, with an increase of about 5 units compared to *CTRL* (p $<$ 0.01), indicating a clear preferential allocation of biomass to the aerial part. In contrast, the other treatments did not significantly alter the ratio.

**7.5.2.3 Plant Morphological and Physiological Traits** Leaves number was significantly affected by the amendment but non by the application rate (Fig.28A). Plants grown with *VERM* showed the highest number of leaves, averaging to $16.0 \pm 1.15$ leaves per plant, i.e. an increase of about 4.5 leaves compared to *CTRL* $11.5 \pm 1.70$ mean leaves; p $<$ 0.05). *COMM*, *INDG* and *PRUN* did not differ from the *CTRL*.

Chlorophyll content, expressed as SPAD index, was not significantly affected by the amendment (Fig. 28B).

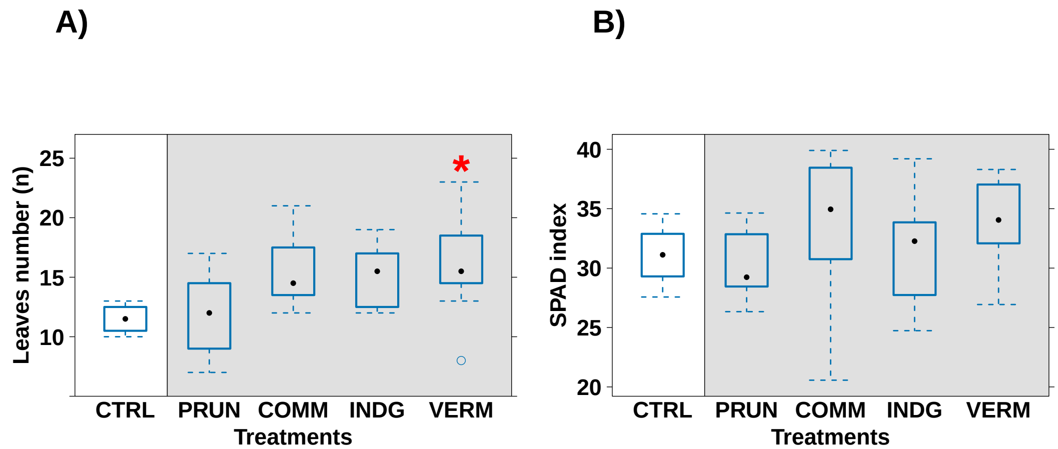


Figure 28: Effects of organic amendments on lettuce morphology and physiology. (A) Leaves number and (B) SPAD index (chlorophyll content) under different amendments. Asterisks indicate statistical significance of pairwise comparisons versus CTRL: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

Similarly, no significant effects of the amendments were detected on the colorimetric parameters (L*, a*, b*, Chroma, Hue angle). All indices showed comparable values across treatments, and significant differences in leaf color were not found (Fig. S26 A-E).

The maximum quantum efficiency of photosystem II (Fv/Fm) was consistently above the reference threshold of 0.83 in all treatments (Fig. S26 F; Table S25), indicating the absence of photoinhibition and suggesting that plants maintained optimal photosynthetic efficiency regardless of the amendment applied.

## 7.6 Discussion

### 7.6.1 Effects of Organic Amendments on Substrate Physicochemical Parameters

The first objective of this study was to evaluate the influence of different organic amendments on substrate physicochemical parameters, with particular attention to substrate water retention and pore water electrical conductivity. Our findings confirmed that both amendment type and dose decidedly shaped substrate water dynamics, with clear contrasts between vermicompost and composts derived from vine pruning.

In both crop phases, *VERM* significantly increased substrate VWC compared to *CTRL*. This pattern is coherent with earlier reports indicating that either poorly stabilized composts, or composts inoculated with microbial consortia, may transiently impair substrate structure and reduce water availability (Zmora-Nahum et al. 2007). Conversely, the improvement observed with *VERM* is consistent with many studies demonstrating that vermicompost enhances porosity, aggregate stability, and water-holding capacity (Chaoui et al. 2003; Edwards et al. 2010; Sanchez-Monedero et al. 2018).

The comparison over time between crop phases further highlights an important dynamic: during the first cropping cycle (2024, *Triticum dicoccum*), significant increases in VWC were observed only at *VERM* doses higher than 16 t $ha^{-1}$; by contrast, in the second cycle (2025, *Lactuca sativa*), positive effects were already evident at the lowest VERM dose (2 t $ha^{-1}$). This suggests a cumulative or additive effect of repeated VERM applications on the same substrate, consistent with long-term studies showing progressive improvement of substrate structure and OM stability as a consequence of successive applications (Diacono and Montemurro 2010; Martínez-Blanco et al. 2013).

Regarding ECp, responses were limited and treatment-specific. All treatments showed only sporadic differences from *CTRL* in ECp values, and effect remained moderate and within agronomically acceptable thresholds, in line with previous work (Atiyeh et al. 2000; Hargreaves et al. 2008; Lazcano and Dominguez 2011).

Notably, continuous monitoring during the second cycle confirmed that *VERM* at 32 t $ha^{-1}$ significantly increased VWC compared to the *CTRL* throughout the experiment, indicating a stable and persistent effect rather than transient fluctuations.

### 7.6.2 Effects of Organic Amendments on Plant Growth and Biomass Allocation

The second objective of this study was to assess whether changes in substrate water status and fertility translated into plant growth and biomass partitioning. Our results highlighted a strong effect of amendments, again with clear differences between vermicompost and pruning-derived ones.

In agreement with previous studies where vermicompost increased growth and productivity in horticultural crops (Arancon et al. 2004; Lazcano and Dominguez 2011), the *VERM* application consistently promoted both higher shoot biomass and leaves number in lettuce, with plants showing on average 4.5 more leaves than *CTRL*. The increase in shoot-to-root ratio further indicates a preferential allocation of resources to aboveground biomass in *VERM* treatments.

By contrast, *COMM* and *INDG* while stimulating root development, did not significantly improved either shoot biomass or leaves number. Similar effects have been reported for composts of limited maturity or containing microbial inoculants, which may favor root exploration without enhancing total plant productivity (Morra et al. 2021; Tognetti et al. 2005). *PRUN* showed no significant effect on any of the measured PP, confirming the limited short-term agronomic value of lignin-rich residues (Poosathit et al. 2024).

### 7.6.3 Physiological Responses and Interpretation of Growth Patterns

The third objective was to evaluate whether effects of amendments on plant growth were associated with changes in leaf physiology, including chlorophyll content and pigmentation. Our results demonstrated that neither SPAD index nor colorimetric traits (L*, a*, b*, Chroma, Hue angle) were significantly affected by the amendments ($p > 0.05$). These indices are widely recognized as proxies for leaf nitrogen status and chlorophyll concentration (Padilla et al. 2018; Richardson et al. 2002; Uddling et al. 2007). Their homogeneity across treatments suggests that nutrient availability - nitrogen in particular - was not the factor explaining growth differences. The Fv/Fm values further support this interpretation. In all treatments they remained consistently above 0.83, the threshold typical of non-stressed plants, indicating the absence of photoinhibition. This evidence reinforces the conclusion that amendments did not alter photosynthetic efficiency but instead influenced growth through effects on water dynamics and biomass partitioning (Baker 2008; Maxwell and Johnson 2000).

The higher shoot biomass and the greater proportion of biomass allocated to shoots observed in plants treated with *VERM* in substrate, is therefore more likely related to a greater water availability, which reduced the need for root investment and enabled plants to prioritize aerial development. This interpretation is consistent with findings by Rouphael et al. (2012) i.e. water supply often has a stronger effect than nutrients on biomass partitioning in leafy vegetables.

### 7.6.4 Agronomic Implications and Perspectives

Our findings confirm the central role of vermicompost in substrate water dynamics and crop performance, even at moderate application rates, suggesting its potential as a sustainable amendment for Mediterranean agricultural systems, where water scarcity is an emerging issue. The moderate increase in ECp observed under *VERM* suggest some extra care when high doses are repeatedly applied, though values remained within safe ranges under our experimental conditions. By contrast, the inconsistent effects of pruning-derived composts (*PRUN*, *COMM*, *INDG*) reflect the variability of such materials, as widely documented in the literature (Morra et al. 2021; Zmora-Nahum et al. 2007). Optimizing their production process, through co-composting, maturation, or blending with more labile substrates, could improve their agronomic value.

Finally, future studies should address long-term dynamics and potential trade-offs, including SOM stabilization, microbial community shifts, and the accumulation of contaminants such as heavy metals or microplastics, increasingly reported in urban-derived composts (Bläsing and Amelung 2018; Porterfield et al. 2023; Weithmann et al. 2018). Integrating these aspects will be essential to fully disclose the sustainability of compost and vermicompost within circular economy frameworks.

## 7.7 Conclusions

This study investigated some short-term effects of different organic amendments on substrate physicochemical properties and lettuce performance under pot conditions. The main objective was to compare pruning-derived composts (*PRUN*, *COMM*, *INDG*), vermicompost from spent coffee grounds (*VERM*) with an unamended control (*CTRL*), and to evaluate their capacity to enhance substrate water retention, salinity, and plant growth.

Our findings clearly showed that the amendment type was the primary factor driving changes on substrate behaviour and plant responses. Vermicompost consistently increased substrate water content, with effects already noticeable at the lowest dose (2 t $ha^{-1}$) in the second crop phase. These results confirm that vermicompost, owing to its fine structure and stable OM, is particularly effective in enhancing substrate water-holding capacity, while pruning-derived composts are less reliable in the short term.

Electrical conductivity data were less clear. Even if *VERM* tended to increase it at high doses, nevertheless the values remained within agronomic thresholds, suggesting limited risk under the tested conditions. Continuous monitoring confirmed the stability of these effects, with *VERM* maintaining higher water content and salinity throughout the cycle.

Physiological traits on plants reflected the substrate dynamics. Vermicompost significantly increased shoot biomass and leaves number, and pushed biomass production toward aboveground tissues, while pruning-derived composts mainly stimulated root growth without translating into greater aerial development. Importantly, physiological indicators such as SPAD index and colorimetric traits did not differ among treatments, suggesting that water availability, rather than nutrient supply, was the main driver of the observed growth advantages in *VERM*-amended plants.

Altogether, this experiment highlights the potential of vermicompost as a sustainable amendment to improve water retention and productivity in Mediterranean systems. However, the inconsistent effects of pruning-derived composts underline the importance of optimizing their production and evaluating longer-term impacts, including SOM stabilization and potential accumulation of contaminants.

## 7.8 References

Altieri MA, Nicholls CI (2017) Agroecology: A brief account of its origins and currents of thought in Latin America. Agroecology and Sustainable Food Systems 41:231–237. https://doi.org/10.1080/21683565.2017.1287147

Arancon NQ, Edwards CA, Bierman P, Welch C, Metzger JD (2004) Influences of vermicomposts on field strawberries: 1. Effects on growth and yields. Bioresource Technology 93:145–153. https://doi.org/10.1016/j.biortech.2003.10.014

Atiyeh RM, Arancon N, Edwards CA, Metzger JD (2000) Influence of earthworm-processed pig manure on the growth and yield of greenhouse tomatoes. Bioresource Technology 75:175–180. https://doi.org/10.1016/S0960-8524(00)00064-X

Baker NR (2008) Chlorophyll Fluorescence: A Probe of Photosynthesis In Vivo. Annual Review of Plant Biology 59:89–113. https://doi.org/10.1146/annurev.arplant.59.032607.092759

Bernal MP, Alburquerque JA, Moral R (2009) Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresource Technology 100:5444–5453. https://doi.org/10.1016/j.biortech.2008.11.027

Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microbial Cell Factories 13:66. https://doi.org/10.1186/1475-2859-13-66

Björkman O, Demmig B (1987) Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 170:489–504. https://doi.org/10.1007/BF00402983

Bläsing M, Amelung W (2018) Plastics in soil: Analytical methods and possible sources. Science of The Total Environment 612:422–435. https://doi.org/10.1016/j.scitotenv.2017.08.086

Chaoui HI, Zibilske LM, Ohno T (2003) Effects of earthworm casts and compost on soil microbial activity and plant nutrient availability. Soil Biology and Biochemistry 35:295–302. https://doi.org/10.1016/S0038-0717(02)00279-1

Diacono M, Montemurro F (2010) Long-term effects of organic amendments on soil fertility. A review. Agronomy for Sustainable Development 30:401–422. https://doi.org/10.1051/agro/2009040

Dominguez J, Edwards C (2010) Relationships between Composting and Vermicomposting. In: Edwards C, Arancon N, Sherman R (eds) Vermiculture Technology. CRC Press, pp 11–25

Edwards CA, Arancon NQ, Sherman RL (eds) (2010) Vermiculture Technology, 0th edn. CRC Press

FAO (2018) The 10 Elements of Agroecology. Guiding the Transition to Sustainable Food and Agricultural Systems.

Feller C, Blanchart E, Bernoux M, Lal R, Manlay R (2012) Soil fertility concepts over the past two centuries: The importance attributed to soil organic matter in developed and developing countries. Archives of Agronomy and Soil Science 58:S3–S21. https://doi.org/10.1080/03650340.2012.693598

Ferreira CSS, Seifollahi-Aghmiuni S, Destouni G, Ghajarnia N, Kalantari Z (2022) Soil degradation in the European Mediterranean region: Processes, status and consequences. Science of The Total Environment 805:150106. https://doi.org/10.1016/j.scitotenv.2021.150106

Hargreaves J, Adl M, Warman P (2008) A review of the use of composted municipal solid waste in agriculture. Agriculture, Ecosystems & Environment 123:1–14. https://doi.org/10.1016/j.agee.2007.07.004

Iglesias A, Garrote L (2015) Adaptation strategies for agricultural water management under climate change in Europe. Agricultural Water Management 155:113–124. https://doi.org/10.1016/j.agwat.2015.03.014

Johnson GN, Young AJ, Scholes JD, Horton P (1993) The dissipation of excess excitation energy in British plant species. Plant, Cell & Environment 16:673–679. https://doi.org/10.1111/j.1365-3040.1993.tb00485.x

Keliikuli A, Smith K, Li Y, Lee CN (2019) Natural Farming: The Development of Indigenous Microorganisms Using Korean Natural Farming Methods

Lal R (2004) Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. Science 304:1623–1627. https://doi.org/10.1126/science.1097396

Lazcano C, Dominguez J (2011) The use of vermicompost in sustainable agriculture: Impact on plant growth and soil fertility. In: Soil nutrients

Lim SL, Wu TY, Sim EYS, Lim PN, Clarke C (2012) Biotransformation of rice husk into organic fertilizer through vermicomposting. Ecological Engineering 41:60–64. https://doi.org/10.1016/j.ecoleng.2012.01.011

Lou XF, Nair J (2009) The impact of landfilling and composting on greenhouse gas emissions – A review. Bioresource Technology 100:3792–3798. https://doi.org/10.1016/j.biortech.2008.12.006

Manlay RJ, Feller C, Swift MJ (2007) Historical evolution of soil organic matter concepts and their relationships with the fertility and sustainability of cropping systems. Agriculture, Ecosystems & Environment 119:217–233. https://doi.org/10.1016/j.agee.2006.07.011

Martínez-Blanco J, Lazcano C, Christensen TH, Muñoz P, Rieradevall J, Møller J, Antón A, Boldrin A (2013) Compost benefits for agriculture evaluated by life cycle assessment. A review. Agronomy for Sustainable Development 33:721–732. https://doi.org/10.1007/s13593-013-0148-7

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. Journal of Experimental Botany 51:659–668. https://doi.org/10.1093/jexbot/51.345.659

Medina J, Monreal C, Barea JM, Arriagada C, Borie F, Cornejo P (2015) Crop residue stabilization and application to agricultural and degraded soils: A review. Waste Management 42:41–54. https://doi.org/10.1016/j.wasman.2015.04.002

Montanarella L, Pennock DJ, McKenzie N, et al (2016) World's soils are under threat. SOIL 2:79–82. https://doi.org/10.5194/soil-2-79-2016

Morra L, Bilotto M, Baldantoni D, Alfani A, Baiano S (2021) A seven-year experiment in a vegetable crops sequence: Effects of replacing mineral fertilizers with Biowaste compost on crop productivity, soil organic carbon and nitrates concentrations. Scientia Horticulturae 290:110534. https://doi.org/10.1016/j.scienta.2021.110534

Najafzadeh R, Arzani K, Bouzari N, Hashemi J (2014) Identification of new Iranian sour cherry genotypes with enhanced fruit quality parameters and high antioxidant properties. New Zealand Journal of Crop and Horticultural Science 42:275–287. https://doi.org/10.1080/01140671.2014.918044

Padilla FM, Gallardo M, Peña-Fleitas MT, De Souza R, Thompson RB (2018) Proximal Optical Sensors for Nitrogen Management of Vegetable Crops: A Review. Sensors 18:2083. https://doi.org/10.3390/s18072083

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012) Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. New Phytologist 193:30–50. https://doi.org/10.1111/j.1469-8137.2011.03952.x

Poosathit R, Kunlanit B, Rasche F, Vityakon P (2024) Different Quality Classes of Decomposing Plant Residues Influence Dissolved Organic Matter Stoichiometry Which Results in Different Soil Microbial Processing. Soil Systems 8:28. https://doi.org/10.3390/soilsystems8010028

Porterfield KK, Hobson SA, Neher DA, Niles MT, Roy ED (2023) Microplastics in composts, digestates, and food wastes: A review. Journal of Environmental Quality 52:225–240. https://doi.org/10.1002/jeq2.20450

R Core Team (2023) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Richardson AD, Duigan SP, Berlyn GP (2002) An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytologist 153:185–194. https://doi.org/10.1046/j.0028-646X.2001.00289.x

Rouphael Y, Cardarelli M, Schwarz D, Franken P, Colla G (2012) Effects of Drought on Nutrient Uptake and Assimilation in Vegetable Crops. In: Aroca R (ed) Plant Responses to Drought Stress. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 171–195

Sanchez-Monedero MA, Cayuela ML, Roig A, Jindo K, Mondini C, Bolan N (2018) Role of biochar as an additive in organic waste composting. Bioresource Technology 247:1155–1164. https://doi.org/10.1016/j.biortech.2017.09.193

Sarkar D (2001) Lattice: Trellis Graphics for R

Smith P, Soussana J-F, Angers D, et al (2020) How to measure, report and verify soil carbon change to realize the potential of soil carbon sequestration for atmospheric greenhouse gas removal. Global Change Biology 26:219–241. https://doi.org/10.1111/gcb.14815

Tognetti C, Laos F, Mazzarino MJ, Hernández MT (2005) Composting vs. Vermicomposting: A Comparison of End Product Quality. Compost Science & Utilization 13:6–13. https://doi.org/10.1080/1065657X.2005.10702212

Uddling J, Gelang-Alfredsson J, Piikki K, Pleijel H (2007) Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynthesis Research 91:37–46. https://doi.org/10.1007/s11120-006-9077-5

Weithmann N, Möller JN, Löder MGJ, Piehl S, Laforsch C, Freitag R (2018) Organic fertilizer as a vehicle for the entry of microplastic into the environment. Science Advances 4:eaap8060. https://doi.org/10.1126/sciadv.aap8060

Wickham H (2016) Tidyverse: Easily Install and Load the 'Tidyverse'

Zmora-Nahum S, Hadar Y, Chen Y (2007) Physico-chemical properties of commercial composts varying in their source materials and country of origin. Soil Biology and Biochemistry 39:1263–1276. https://doi.org/10.1016/j.soilbio.2006.12.017

## 7.9 Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## 7.11 Author contributions

Francesco Serafini: Conceptualization; Investigation; Formal Analysis; Writing – Original Draft Preparation.

Ottorino-Luca Pantani: Methodology; Investigation; Formal Analysis; Writing – Review & Editing.

Marco Nocentini: Investigation; Writing – Review & Editing.

Lorenzo Ferretti: Investigation; Writing – Review & Editing.

Gaio Cesare Pacini: Conceptualization; Supervision; Project Administration; Writing – Review & Editing.

Anita Maienza: Validation; Formal Analysis; Writing – Review & Editing.

Margherita Santoni: Methodology; Investigation; Formal Analysis; Writing – Review & Editing.

All authors have read and approved the final version of the manuscript.

## 7.12 Supplementary Materials

Table S22: Main physico-chemical characteristics of soil from the Montepaldi Long Term Experiment.

| Parameter | Value |
|---|---|
| Sand (%) | 17.6 |
| Silt (%) | 49.4 |
| Clay (%) | 33.0 |
| Texture | Silty clay loam |
| pH ($H_2O$) | 8.3 |
| CEC ($cmol^+ \ kg^{-1}$) | 18.2 |
| Total $P_2O_5$ (%) | 1.66 |
| Available $P_2O_5$ ($mg \ kg^{-1}$) | 27 |
| Exchangeable $K_2O$ ($mg \ kg^{-1}$) | 143 |

Table S23: Volumetric water content of substrate (VWC, %, mean ± SE) in the two crop phases (2024, *Triticum dicoccum*; 2025, *Lactuca sativa*) under different organic amendments. Asterisks indicate significant differences from CTRL ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

| | CTRL | PRUN ($t\,ha^{-1}$) | | | | COMM ($t\,ha^{-1}$) | | | | INDG ($t\,ha^{-1}$) | | | | VERM ($t\,ha^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | 0 | 2 | 8 | 16 | 32 | 2 | 8 | 16 | 32 | 2 | 8 | 16 | 32 | 2 | 8 | 16 | 32 |
| 2024-07-01 | 18.3 (0.58) | 17.5 (0.64) | 19.0 (0.55) | 17.8 (0.94) | 19.6 (1.19) | 18.4 (1.30) | 17.4 (1.02) | 17.0 (0.62) | 15.3 (0.83) * | 17.4 (0.79) | 16.6 (0.86) | 13.9 (1.02) *** | 16.1 (0.62) | 18.4 (0.88) | 19.0 (0.44) | 20.3 (0.75) | 22.4 (1.04) *** |
| 2024-07-08 | 12.4 (0.40) | 11.8 (0.65) | 12.0 (0.95) | 11.0 (0.65) | 13.6 (0.87) | 11.4 (0.47) | 11.3 (0.56) | 11.6 (0.63) | 9.7 (0.61) ** | 9.9 (0.71) ** | 10.0 (0.88) * | 10.1 (0.58) ** | 10.9 (0.76) | 12.5 (0.63) | 12.5 (0.73) | 14.4 (0.51) * | 15.9 (1.08) *** |
| 2024-07-17 | 13.3 (0.31) | 12.6 (0.73) | 12.4 (1.11) | 12.6 (0.87) | 15.4 (0.86) | 12.7 (0.50) | 12.0 (0.81) | 12.2 (0.61) | 10.3 (0.43) *** | 10.8 (0.49) *** | 10.9 (0.59) ** | 10.2 (0.47) *** | 11.2 (0.69) * | 12.3 (0.55) | 13.9 (0.67) | 14.4 (0.72) | 17.3 (1.36) *** |
| 2024-07-24 | 7.9 (0.34) | 7.0 (0.42) | 6.8 (0.75) | 6.7 (0.82) | 8.7 (1.43) | 8.0 (0.82) | 7.7 (0.66) | 6.7 (0.36) | 7.4 (0.48) | 6.5 (0.45) | 6.3 (0.76) | 5.7 (0.36) * | 5.8 (0.61) * | 7.6 (0.69) | 8.3 (0.95) | 10.0 (1.29) * | 10.8 (1.28) *** |
| 2025-07-21 | 9.1 (0.85) | 11.4 (1.36) | 8.2 (1.62) | 9.6 (1.20) | 10.4 (2.86) | 9.5 (0.79) | 10.7 (5.35) | 10.5 (1.45) | 9.1 (0.84) | 10.7 (2.45) | 10.7 (1.50) | 9.8 (0.73) | 11.9 (1.66) | 13.7 (1.34) ** | 14.1 (1.57) * | 13.0 (0.98) * | 14.8 (1.43) ** |
| 2025-07-28 | 8.1 (0.51) | 10.3 (0.56) * | 8.4 (1.24) | 10.6 (0.40) | 8.5 (0.85) | 8.6 (1.20) | 9.1 (2.30) | 9.4 (0.26) | 5.7 (1.10) * | 9.0 (1.02) | 9.0 (0.73) | 8.1 (0.72) | 10.5 (0.71) * | 11.8 (0.51) *** | 10.2 (0.81) | 10.3 (1.46) * | 12.0 (1.21) ** |

Table S24: Pore water conductivity of substrate (ECp, mS $m^{-1}$, mean ± SE) in the two crop phases (2024, *Triticum dicoccum*; 2025, *Lactuca sativa*) under different organic amendments. Asterisks indicate significant differences from CTRL ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

| | CTRL | PRUN ($t\,ha^{-1}$) | | | | COMM ($t\,ha^{-1}$) | | | | INDG ($t\,ha^{-1}$) | | | | VERM ($t\,ha^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | 0 | 2 | 8 | 16 | 32 | 2 | 8 | 16 | 32 | 2 | 8 | 16 | 32 | 2 | 8 | 16 | 32 |
| 2024-07-01 | 110.0 (1.38) | 108.0 (2.07) | 104.9 (1.46) | 109.2 (2.07) | 107.6 (1.15) | 121.5 (11.81) * | 105.0 (1.42) | 108.2 (4.23) | 106.7 (1.65) | 104.5 (2.00) | 112.1 (6.52) | 113.6 (3.65) | 105.4 (1.60) | 105.0 (1.37) | 112.4 (2.86) | 116.1 (4.79) | 118.2 (3.62) ** |
| 2024-07-08 | 99.4 (2.00) | 92.4 (2.88) | 99.5 (5.66) | 99.1 (4.14) | 100.7 (3.47) | 101.1 (2.56) | 93.8 (2.19) | 90.0 (2.48) * | 98.5 (4.80) | 88.2 (2.08) * | 90.8 (5.03) | 88.7 (2.99) * | 94.7 (2.12) | 90.6 (2.34) * | 102.2 (5.00) | 101.3 (2.16) | 108.4 (4.10) * |
| 2024-07-17 | 109.4 (1.94) | 102.1 (1.70) | 105.8 (1.66) | 107.2 (1.62) | 105.6 (3.53) | 109.8 (4.26) | 101.5 (4.35) * | 98.2 (1.53) ** | 103.5 (1.83) | 105.1 (2.01) | 101.6 (2.94) | 101.9 (3.33) | 101.1 (1.70) * | 102.8 (3.55) | 107.4 (2.24) | 108.0 (4.25) | 114.3 (3.20) |
| 2025-07-21 | 133.7 (7.16) | 132.3 (4.83) | 121.2 (8.60) | 127.0 (6.63) | 120.0 (NA) | 144.8 (18.14) | 136.4 (NA) | 132.7 (2.72) | 141.6 (12.20) | 120.0 (3.73) | 111.1 (6.89) | 133.8 (4.20) | 122.9 (4.38) | 125.3 (6.33) | 124.3 (4.56) | 122.0 (2.30) | 125.1 (2.64) |
| 2025-07-28 | 136.4 (5.17) | 137.9 (4.96) | 137.5 (5.82) | 134.7 (3.99) | 133.7 (1.20) | 129.6 (5.56) | 123.2 (NA) | 131.8 (5.03) | NA (NA) | 129.1 (3.55) | 125.6 (5.98) | 132.0 (8.83) | 130.1 (2.98) | 132.1 (3.96) | 130.9 (3.17) | 128.3 (5.05) | 131.7 (3.61) |

Table S25: Results of one-sample t-tests comparing Fv/Fm values of lettuce plants with the theoretical optimal threshold of 0.83. Values are reported as treatment means, their difference from 0.83 and the associated p-value.

| Treatment | Mean Fv/Fm | Difference from 0.83 | p-value |
|---|---|---|---|
| CTRL | 0.847 | +0.017 | 0.0073 |
| PRUN | 0.858 | +0.028 | $< 10^{-3}$ |
| COMM | 0.856 | +0.026 | $< 10^{-3}$ |
| INDG | 0.856 | +0.026 | $< 10^{-3}$ |
| VERM | 0.859 | +0.029 | $< 10^{-3}$ |

Table S26: Paired t-test results comparing VERM32 and CTRL treatments for daily substrate volumetric water content (VWC, %) during the second crop phase (2025, *Lactuca sativa*).

| Parameter | Comparison | t (df) | Mean difference | 95% CI | p-value |
|---|---|---|---|---|---|
| VWC (%) | VERM32 − CTRL | 198.93 (9828) | +3.6 | +3.40 to +3.84 | $< 10^{-3}$ |

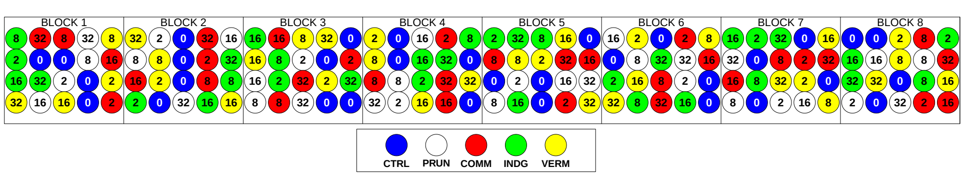


Figure S23: Graphical representation of the completely randomized blocks experimental design. Numbers within blocks indicate the assigned dose for each treatment. Colours indicate the amendments as in legend.


Figure S24: Instruments used for substrate and plant measurements during the experiment: HH2 Moisture Meter (Delta-T Devices, Cambridge, UK) for substrate volumetric water content (VWC) and pore water electrical conductivity (ECp); SPAD-502Plus meter (Konica Minolta, Osaka, Japan) for leaf chlorophyll content; and Handy PEA+ fluorimeter (Hansatech Instruments, Norfolk, UK) for chlorophyll fluorescence (Fv/Fm).

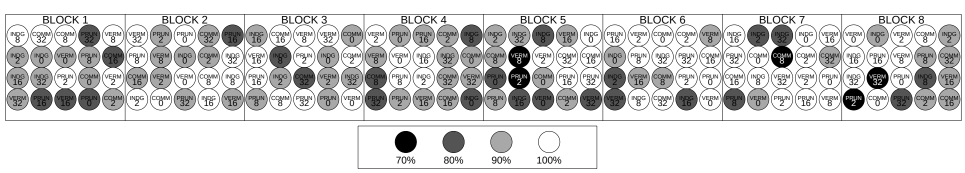


Figure S25: Graphical representation of Triticum dicoccum germination rates under different treatments (CTRL, PRUN, COMM, INDG, VERM) and application rates (2, 8, 16, 32 t ha$^{-1}$). Circles indicate individual pots (n = 160), arranged in eight completely randomized blocks. Shading intensity corresponds to germination success (70%, 80%, 90%, 100%), as detailed in the legend.

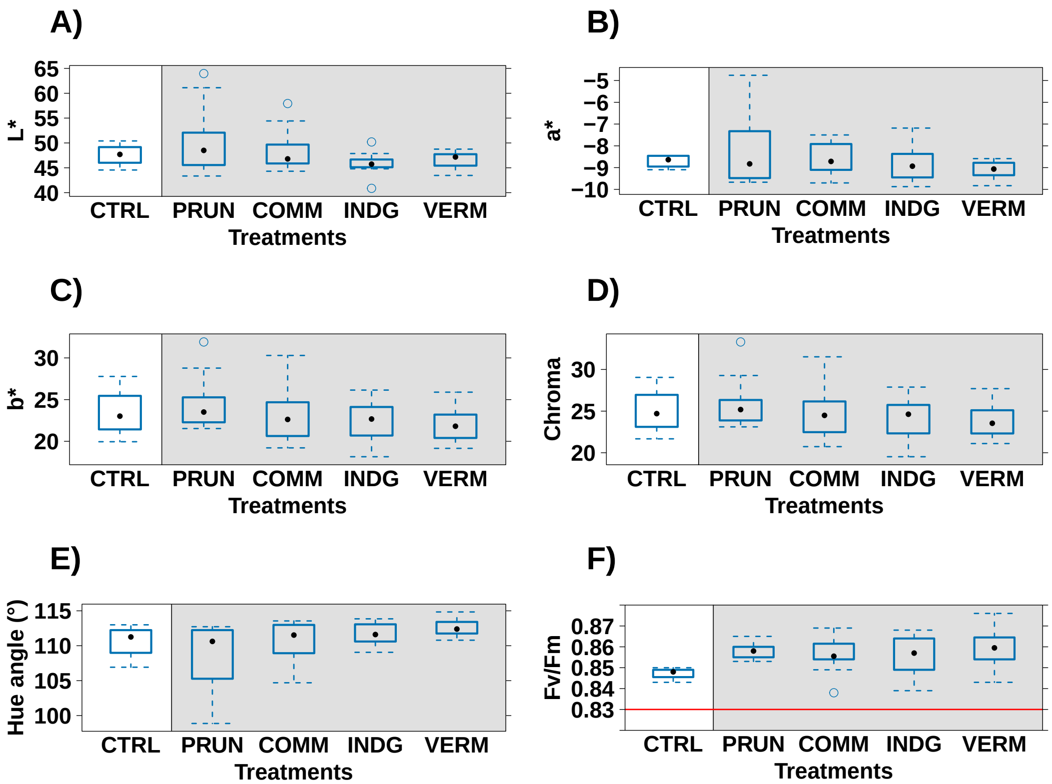


Figure S26: Effects of organic amendments on leaf colorimetric parameters and photosystem II efficiency. (A) Lightness (L*), (B) green–red component (a*), (C) yellow–blue component (b*), (D) Chroma, and (E) Hue angle, and (F) maximum quantum efficiency of PSII (Fv/Fm) in lettuce leaves under different treatments. Asterisks indicate statistical significance of pairwise comparisons versus CTRL: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). The red dashed line in panel (F) represents the reference threshold for unstressed plants.

# 8 Main Conclusions

The main objective of this work was to evaluate the potential of agroecological practices and soil management within the constraints of circular economy and organic farming to enhance fertility, crop productivity, and energy use efficiency in Mediterranean arable systems. Five specific goals were pursued:

- to assess long-term yield and energy use efficiency trends in organic and conventional farming systems under Mediterranean conditions;
- to summarize the scientific evidences on the agronomic impacts of Municipal Solid Waste Compost (MSWC);
- to test repeated applications ad realistic yet economically viable amounts of vine pruning composts and vermicompost in a long-term experiment on organic farming;
- to characterize the behavioral and functional responses of the endemic earthworm *Hormogaster samnitica* to the same amendments in mesocosm conditions.
- to investigate short-term, dose-dependent effects of the amendments previous cited in controlled pot trials;

The following conclusions can be drawn:

- Long-term analysis at the Montepaldi Long Term Experiment (MoLTE) demonstrated that organic systems produced lower yields (≈-21% in winter cereals) but exhibited markedly higher energy use efficiency (≈+33%), confirming that organic farming represents a viable option to address the "Perfect Storm" of food, water, and energy insecurity in the Mediterranean context. Spring crops, however, showed dramatic yield declines in both systems over the last 30 years, driven by reduced rainfall and increased temperatures, pointing to climate change as the overriding constraint.
- The systematic review of Municipal Solid Waste Compost (MSWC) studies confirmed its agronomic potential, particularly at high application rates ($> 50$ t ha$^{-1}$), where consistent positive effects on yield and soil organic matter were found. However, variability in either compost composition, or crop-specific responses, or concerns regarding contaminants (heavy metals, microplastics) highlights the need for stricter quality control and long-term field validation before MSWC can be fully integrated into sustainable strategies.

- Field experiments with repeated realistic yet economically viable inputs (2 t $ha^{-1}$ $yr^{-1}$) indicated modest but cumulative benefits: pruning-derived composts and vermicompost improved early-time aggregate stability while crop yield improvements emerged only after two years. Vermicompost also increased earthworm biomass. These findings suggest that even small, sustained applications can contribute to soil function and productivity, but they must be coupled with nutrient management, particularly for phosphorus.

- Mesocosm trials with *Hormogaster samnitica*, revealed earthworm size-dependent functional roles: larger individuals create wider and deeper burrows thus enhancing macroporosity and water infiltration, while juveniles ones exerted limited influence. Compost-based amendments increased macropore formation in the amended layer and were generally more attractive to earthworms than vermicompost, although these structural changes did not always translate into higher infiltration rates. These results emphasize the relevance of soil fauna traits in mediating amendment effects.

- Pot experiments highlighted the decisive role of amendment type and dose. Vermicompost consistently improved substrate water retention, shoot biomass, and leaf production in lettuce, while pruning-derived composts produced limited or inconsistent effects, mainly stimulating root growth without enhancing aboveground performance. These results underline the importance of amendment quality and composition in shaping short-term plant–soil interactions.

In conclusion, this thesis confirms that Mediterranean agriculture faces three interlinked challenges: (i) producing sufficient food under resource constraints, (ii) maintaining productivity with lower energy inputs, and (iii) enhancing resilience to water scarcity. Organic farming, supported by wastes from circular economy, can partially address these challenges by improving soil biological activity, water relations, and energy use efficiency. However, the persistence of yield gaps between organic and conventional farming systems, the site- and crop-specific nature of amendment effects, and unresolved concerns about compost quality, point to the need for further research. Future efforts should prioritize: (i) developing resilient crop varieties suited to organic systems under climate stress, (ii) optimizing amendment production and application strategies, and (iii) deepening the integration of soil fauna into soil fertility assessments.

# 9 Supplementary Materials

## 9.1 FunBies, a Model for Integrated Assessment of Functional Biodiversity of Weed Communities in Agro-ecosystem

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### 9.1.1 Abstract

Agrobiodiversity, by producing beneficial ecosystem services (ESs), could improve the sustainability of cropping systems. There is a number of studies reporting the use of indicators for quantifying ESs. However, there are no indicators which might be applied at local scale and allowing an integrated assessment of a wide range of ESs in agro-ecosystems. The objectives of the present research were: (i) to describe a model for integrated assessment of functional biodiversity in agro-ecosystems, denominated FunBies, (ii) to show how it was validated, and (iii) to present results of its application. FunBies is featured by an empiric model component, a conceptual component that takes into account the whole range of ESs identified by the Millennium Ecosystems Assessment and by a multi-criteria linear additive model including the whole set of functional traits potentially supplied by herbaceous plant communities. The model was validated by a panel of experts. Results at cropping system level indicated that organic systems have the potential to supply considerably higher ESs than conventional systems. ES provision increases in time together with the evolution of the phytocoenosis. FunBies potential applications include: (i) design of biodiversity components within agro-ecosystems, and (ii) justification and sizing of organic payments.

### 9.1.2 Keyword

Functional Biodiversity, Integrated assessment, Functional traits, Weed community, Organic farming

**9.1.2.1 List of acronyms** FunBies, functional biodiversity of agro-ecosystems

OO, old organic

NO, new organic

CO, conventional

RC, row crop

WC, winter cereal

LF, legume crop for forage

LG, legume crop for grain

ES, ecosystem service

EF, ecosystem function

FT, functional trait

MVA, multivariate analysis

FBI, functional biodiversity index

MoLTE, Montepaldi long term experiment

MA, Millennium Ecosystem Assessment

TEEB, The Economics of Ecosystems and Biodiversity

### 9.1.3 Introduction

The concept of functional biodiversity has been introduced to acknowledge the fact that the components of biological diversity are not only important per se but also for the ecosystem functions (EFs) they supply. The importance of ecosystem functions was streamlined in the mid-sixties, has been progressively acknowledged during the nineties and gained global attention after the publication of the Millennium Ecosystem Assessment (MA) Reports (2005).

De Groot (1992) defined ecosystems functions as “the capacity of natural processes and components to provide goods and services that satisfy human needs, directly or indirectly”. Coherently, ecosystem services (ESs) were later defined as the benefits that people derive from ecological functions of ecosystems (Costanza and Folke 1997; Millennium Ecosystem Assessment 2005). The link between ecosystems functions and biodiversity in agro-ecosystems was explicated by the definition of functional biodiversity given by Moonen and Barberi (2008), i.e. “that part of the total biodiversity composed of clusters of elements (at the gene, species or habitat level) providing the same (agro)ecosystem service, that is driven by within-cluster diversity”.

Costanzo and Barberi (2014) stated that agrobiodiversity, by producing beneficial services, could improve the sustainability of cropping systems in a context of low external inputs and unpredictable climate change. In the MA (2005) ESs were listed and the importance of considering ESs in agroecosystems analysis was stressed. More recently, Costanza et al. (2017) further confirmed the importance of ESs and estimated the value of ESs as 33 trillion ($10^{12}$) \$/year. In addition, they stressed the crucial importance of giving a value for understanding, comparing and quantifying the economic contribution of ES provision.

In this scenario the scientific community plays a fundamental role. It can provide tools and models to evaluate the whole range of ESs (Millennium Ecosystem Assessment 2005) provided by an (agro)ecosystem. Furthermore, tools and models provided by scientific community are crucial to be integrated in an ecological-economical approach; policy measures should be developed including ES provision by using modeling as a tool to develop a full cost accounting which considers negative and positive impacts on ESs and disservices. In this regard, integrated modelling becomes essential to manage economic development in line with the ecological economics approach (Costanza et al. 2017).

This concept is further confirmed on farm and lower scales by Pacini et al. (2015) who developed a model to quantify the impact of organic and conventional farming practices on a number of ecosystem services and disservices ranging from biodiversity provision, to soil erosion, nitrogen and pesticide pollution. The model was then used to evaluate and size agri-environmental measures under the shape of organic payments to remunerate farmers for actual provision of ESs or decrease of impacts on disservices, later adopted by Tuscany Government for the implementation of the Regional Rural Development Plan (2015).

There is a number of studies reporting the use of indicators for quantifying ESs (Egoh et al. 2012). According to The Economics of Ecosystems and Biodiversity (TEEB) initiative (Ring et al. 2010), an indicator serves to indicate or give a suggestion of something of interest and is derived from measures. Oikonomou et al. (2011) proposed a conceptual framework that combines ecosystem function analysis, multi-criteria evaluation and social research methodologies for introducing an ecosystem, function-based planning and management approach.

Egoh et al. (2007) made a literature review about existing ES indicators. In his review, he found that there are several studies which evaluated the ES provision of systems on different scales but not enough research on site scales and in particular on productive farming systems. Bagstad et al. (2013) showed 17 tools to evaluate and quantify ESs. 15 out of 17 can be used at landscape scale and only 2 (EcoMetrix and LUCI) on site scale. EcoMEtrix can be applied to estimate the environmental credits for market-based trading under restoration scenarios, proving that ecosystem functional performance changes depending on changes in attributes. While LUCI can be applied to evaluate land cover change on flood risk, habitat connectivity, erosion, carbon sequestration and agricultural productivity. Therefore, they do not include a wide range of ESs. Egoh et al. (2012) made a review of indicators for mapping ESs from worldwide but in the review, there are no indicators which might be applied at local scale and including a wide range of ESs. In addition, no ES indicators were applied in Italy.

The demand for ecosystem services is increasing in many European countries, yet there is still a scarcity of data on values on regional scale (Gatto et al. 2013). As a result, proxy indicators are often used as surrogates. Proxy methods are especially used for cultural services, as these services are difficult to directly measure and model (Chatzinikolaou et al. 2015). Our concern was to assess the ability of different agro-ecosystem management options to supply ESs, while considering site-specific production and pedo-climatic conditions in a detailed fashion. For this exercise to be effective a measure unit of functional biodiversity is needed that can evaluate the combined impacts of farming practices and the environment on ES provision.

We propose plant functional traits (FTs) as indicators to quantify ESs in agro-ecosystems at a very local scale and under different management options. There are existing studies on the response of functional traits of plant communities to changes caused by external (biotic or abiotic) factors. Lavorel and Garnier (2002) proposed a conceptual framework that links traits associated with responses to those pressures that determine effects on ecosystems. The aim was to integrate analyses of response traits in relation to environmental and/or biotic factors with analyses of functional effects of species, and hence trait composition, in order to analyze the effects of environmental changes on ecosystem processes. Diaz et al. (2004) stated that FTs can be used as predictors of resource capture and utilization which are key-factors for ecosystem functions as a response to climate change and land use. "Through investigations in various parts of the world (Ackerly 2003; Chapin III et al. 1996; Craine et al. 2001; Cunningham et al. 1999; Diaz and Cabido 1997; Grime et al. 1997; Reich et al. 1997; Wardle 1998; Wright et al. 2002) evidence is growing that such predictors do exist, and can be found in the form of single traits or sets of co-occurring traits of plants".

The concept of plant functional type proposes that species can be grouped according to common responses to the environment and/or common effects on ecosystem processes. However, the knowledge of relationships between traits associated with the response of plants to environmental factors such as resources and disturbances (response traits), and traits that determine effects of plants on ecosystem functions (effect traits), such as biogeochemical cycling or propensity to disturbance, remains rudimentary (Lavorel and Garnier 2002). Concerning this last point, we imagine that a modelling tool developed to carry out integrated assessment of a broad range of plant responses and effects can be able to support more refined analyses of functional biodiversity in agro-ecosystems.

The trait-based approach shows promising results, especially for plant trait effects on primary production and some processes associated with carbon and nitrogen cycling in grasslands. However, there is a need to extend the proof of concept for a wider range of ecosystems and ecosystem services and to incorporate not only the functional characteristics of plants but those of other organisms with which plants interact for the provision of ecosystem services Lavorel (2013).

More specifically, based on a review of a number of studies, Lavorel (2013) identified a set of key conceptual and methodological, cross-cutting issues that should be considered for optimizing trait-based assessment of functional biodiversity. Among those, we isolate three issues that we consider particularly important for integrated assessment in agro-ecosystems:

1. The relevance of the "plant economics spectrum" (Freschet et al. 2010) rather than just the leaf economics spectrum (Wright et al. 2004), to ecosystem service provision.

2. Although carbon and nutrient cycling processes are primarily driven by traits of the most

abundant (dominant) species (i.e. the biomass ratio hypothesis; (Grime 1998)), there is new evidence for more complex effects of heterogeneous trait values between species (i.e. functional divergence or niche complementarity).

3. There is also new evidence for the relevance of trait-based analyses of ecosystem services that are underpinned by interactions between plants and, for instance, soil microorganisms or insects (Lavorel et al. 2009).

Weeds have an important role in maintaining farmland biodiversity. This needs to be balanced with their potential negative impact on crop yield and quality (Esposito et al. 2023). Models of crop–weed competition are an important tool in striking this balance (Storkey 2006). As indicated by Moonen and Barberi (2008), we need to consider all the elements composing the productive sub-system in its heterogeneity and not only the semi-natural sub-system where biodiversity conservation is usually focused.

As previously mentioned, Oikonomou et al. (2011) proposed a conceptual, multi-criteria evaluation framework for introducing an ecosystem function-based planning and management approach. However, to our knowledge nobody has applied a multi-criteria approach to assess the impact of alternative farming practices on the capacity of weed communities to produce ESs in agro-ecosystems.

The objectives of the present research were three-fold: i) to describe a model for integrated assessment of functional biodiversity of weed communities in agro-ecosystems, henceforward denominated FunBies (i.e., FUNctional BIodiversity of agro-EcoSystems) model, ii) to show how it was validated, and iii) to present results of its application for the quantification of ESs delivered by weed communities of organic vs conventional systems.

Because mechanistic models of weed community are not developed to the extent needed for our purpose, we built the FunBies model based on empiric evidence from databases of weed communities of cultivated field and semi-natural habitats belonging to Montepaldi long term experiment (MoLTE) were organic and conventional agro-ecosystem management options are compared since 1991.

FunBies is featured by a conceptual component that takes into account the whole range of ESs identified by the MA and by a multi-criteria linear additive model including the whole set of functional traits potentially supplied by herbaceous plant communities representative of cereal, row crop, grain and forage legume fields and semi-natural habitats of Tuscany inland hill, arable land. The model was validated by a panel of experts with reference to pedo-climatic conditions of the area.

### 9.1.4 Material and Methods

**9.1.4.1 Experimental site: The Montepaldi Long Term Experiment** The research took place in the context of MoLTE experimental fields (MoLTE), which are part of an ongoing project started in 1991 at the Department of Agricultural, Food, Environmental and Forestry Sciences, University of Florence (UNIFI-DAGRI). MoLTE fields take place in the experimental farm of Florence University, which is located in Montepaldi, San Casciano Val-di Pesa, Tuscany, Central Italy, and cover an area of about 15 ha, in a lightly sloped area. MoLTE can be considered as a model of a representative agro-ecosystem of the Chianti area and more in general of internal hill arable land of Tuscany.

The experimental site is composed by three differently managed systems, designed with the purpose of comparing organic and conventional management. There are two organically managed systems called “Old Organic” (OO) and “New Organic” (NO) of 5,2 hectares each, composed by 4 fields each, and one “Conventional” system (CO) of 2.6 ha, composed by 2 fields. The two organic systems differ between each other in the time they were converted into organic agriculture. The OO micro-agroecosystem has been converted into organic in 1991 (EC reg. 2092/91 and following regulations), while the NO has been managed under the integrated agriculture method in the period 1991–2000, since 1994 following integrated production rules as indicated by Tuscany Regional implementation program of EC regulation 2078/92, and converted into organic management in 2001. The conventional micro- agroecosystem has been conducted according to ordinary, region- specific, conventional operations, including weeding, fertilization and tillage interventions as illustrated in Appendix A, Table S30.

Organic and conventional micro-agroecosystems include semi-natural habitats composed by an artificial hedgerow composed by autochthonous species (OO boundary), a spontaneous hedgerow (OO-NO) and a spontaneous grass stripe (NO-CO).

**9.1.4.2 Database: observation over 25 years** Spontaneous species data of abundance and biomass has been recorded for MoLTE from 1993. Therefore, a 25-year-old database has been created including 223 records of the spontaneous species collected within the organic and conventional fields with the same method. Further records are available for FunBies concerning biodiversity of semi-natural habitats, which are not considered for the present article devoted to crop-weed communities. In-field weed measurements were based on sampling field portions of 0.25 $m^2$ following the throwing of a square metal sampling frame across the 50 x 260 m fields. Depending on the target number of repeated measurements for each crop in that year, the field was partitioned into equal segments and then the frame was thrown randomly within that segment. All weeds found within the perimeter of the frame were carefully removed, if possible with the root intact, and placed inside a plastic bag. Samples were then transported to the lab where weeds were grouped according to species, and the number of individuals for each species was recorded. The samples were then dried (if fresh weight at species level >0.5 g) and the dry weight per species was recorded. Timing of weed sampling was primarily driven by the combination of three conditions: (i) potential presence of flowering plants to facilitate weed species identification, which mostly happens under local climatic conditions in April-June; (ii) crop-specific phenological phase facilitating weed species identification, which is April-May for winter crops and May-June for summer crops; and (iii) distance from agronomic operations damaging weed species such as mowing of alfalfa or mechanical maize hoeing. Crops are sampled once a year following the calendar reported above, while semi-natural habitats are sampled twice in April and June (Appendix A, Table S31).

**9.1.4.3 Selection of most representative crop-weed communities** In order to quantify ES provision through a functional trait-based approach and to support the assumption that the FunBies model would be able to measure functional biodiversity of alternative management options in Tuscany inland hill arable land, we needed to consider typical community compositions of a broad range of crops under organic and conventional management systems. This was carried out by elaborating a set of 223 samples of crop-weed communities collected over the last 25 years from OO, NO and CO fields of MOLTE with statistical, non-parametric multivariate analysis (MVA) techniques.

MVA statistics allow analyzing correlations between more than one statistical variable at a time, aiming at analyzing the differences between and within groups of samples (Schervish 1987). Each sample was labelled in such a way that it included information of the sampling period, the field and crop in which it was collected and the position within the transect. MVA variables were given by herbaceous plant species collected in the experimental field at each sampling event.

The aim of MVA in our modelling approach was to develop virtual, representative weed communities for both organic and conventional rotations typical of Tuscany inland hill arable land; the species composition of virtual, representative communities would form the database on which subsequently develop a multi-criteria linear additive model for a trait-based, integrated assessment of functional biodiversity.

Typical rotations in our reference period differ between conventional and organic systems, mainly due to the need to include legume crops in organic rotations. Typical conventional rotations last two years and are featured by a row crop followed by a winter cereal. Typical organic rotations last 4 years and include, in addition to row crops and cereals, also legume crops for grain and for forage. In our experiment, row crops (RC) were sunflower (*Helianthus annuus* L.) and maize (*Zea mays* L.); winter cereals (WC) were durum wheat (*Triticum durum* L.), common wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.); legumes for grain (LG) were broad bean (*Vicia faba mino*r L.), lentil (*Vicia lens* (L.) Coss. and Germ.), chickpea (*Cicer arietinum* L.); legumes for forage (LF) were Lucerne (*Medicago sativa* L.) and Clover (*Trifolium squarrosum* L., *T. pratense* L., *T. alexandrinu*m L.).

The final aim of this step was then to identify virtual, representative crop-weed communities for RC, WC, LG and LF crop categories of typical organic and conventional rotations of the reference area. These was achieved by analyzing within the OO, NO and CO sample sets the degree of similarity among corresponding herbaceous plant species, by grouping RC, WC, LG and LF crop samples according to similarity degree, and then by selecting the within-group most representative sets of species. These sets are the ones that we reasonably suppose supporting the provision of ecosystems services from agro-ecosystems of the area. To obtain the composition of OO, NO and CO representative crop-weed communities non-parametric MVA procedures were performed with the software PRIMER 6 (Gorley and Clarke 2006).

First, a similarity matrix which shows the degree of resemblance between each pair of OO, NO and CO sample individuals was calculated using the Bray–Curtis distance (a non-metric coefficient particularly common in ecology, (Bray and Curtis 1957). The resemblance matrix was used as a basis to create a two-dimensional multi-dimensional scaling (MDS) plot for each system (OO, NO and CO), where relative distances of one sample to another represented between-sample (dis)similarities. There is normally some distortion in the plot that is minimized by the MDS algorithm, which is captured by the stress value. The stress value is a goodness-of-fit measure depending on the difference between the distances of each couple of sample points on the MDS plot and the distance predicted from the fitted regression line corresponding to coefficients of dissimilarities. If such difference is equal to zero, the stress is zero. Instead, widely scattered points clearly lead to a large stress and this can be interpreted as measuring the difficulty involved in compressing the sample relationships into two dimensions. Groups of sample individuals were further distinguished by superimposing on the MDS plots graphical representations of cluster analysis (CA) at a chosen similarity level, which is a graphical facility of PRIMER (Clarke and Warwick 2001). Such choice was handled with a heuristic procedure through a subjective inspection of the CA dendrogram (Köbrich et al. 2003).

**9.1.4.4 Characterization of selected crop-weed communities** The similarity percentages (SIMPER) analysis of the sample groups (Clarke 1993) was performed to highlight the species principally responsible for determining the similarities within the crop-weed community groups generated by superimposing MDS and CA.

The SIMPER algorithm first computes the average similarity between all pairs of sample units within a group and then disaggregates this average into separate contributions from each variable. The variables whose values are all equal to zero within a group, although equal, do not give any contribution to the within-group similarity. The rate between within-group similarity and each variable's standard deviation holds a strong characterization power if the variable values are relatively constant within a group, so that standard deviation of its contribution is low, and the ratio between within-group similarity and standard deviation is high.

Species which contributed most to form the groups according to SIMPER analysis were emphasized to characterize each group of samples under OO, NO and CO agro-ecosystem management options, respectively, and were considered for following attribution of ES potentials.

**9.1.4.5 FunBies model** FunBies is a model for integrated assessment of functional biodiversity of weed communities in agro-ecosystems. It is composed by three parts, i.e. an empirical-statistical, crop-weed community component, which is populated by data collected in field and processed with MVA techniques as showed in previous sections, a trait-based conceptual model, which is presented in this section, and a linear additive multi-criteria (LAM) model for integrated assessment of functional biodiversity, which is reported in the next.

ESs are commonly grouped into four categories, depending on corresponding categories of the functions that provide them: provisioning, regulating, cultural and supporting (Millennium Ecosystem Assessment 2005). In our study, we developed a conceptual model which includes all the categories of ESs, in order to quantify the overall ES value provided by crop-weed communities in agro-ecosystems. As the cultivated crops and corresponding spontaneous herbaceous species are typical of the reference area, the model we propose was developed to be valid for the sub-region named "Internal Hill Arable Land" of Tuscany.

For each ES category, we first selected from the MA (2005) and De Groot's (2010) lists ecosystem functions according to their ability to provide target services, relevance for our study and information availability on plant trait databases such as TRY, Ecoflora, BiolFlor and LEDA. Second, a trait-based approach was adopted for evaluating the contribution of each plant to the performance of each function (2013; Pakeman et al. 2011). For this scope, plant functional traits associated with the selected EFs are shown below for each ES category together with corresponding data sources. EFs, FTs, (dis)services and corresponding descriptions are summarized for each of the Millennium Ecosystem Assessment EF categories in Appendix B (online).

The overall conceptual model, including ES categories, specific EFs, corresponding FTs and the way in which are linked is shown in Figure S33 combined with figures resuming the LAM model.

**9.1.4.5.1 Provisioning services** For this ES category only dis-services provided by weeds are considered. Indeed, weeds compete for water, nutrients and other resources with the main crop (Zhang et al. 2007). Whether weeds are more competitive, they both enhance their biomass while reducing the performance of other plants (including the main crop, (Torner et al. 2000). Similarly, several crop parameters (height, yield, biomass) are negatively related to weed biomass (Aminpanah 2013; Power 2010). Therefore, competitiveness is considered to produce a dis-service. According to Torner et al. (2000), plant FTs which better explain the competitive ability of weeds are directly related with plant biomass, plant height, seed weight and rate of emergence. However, after valuation of local experts this set was slightly modified and complemented with additional FTs.

**9.1.4.5.2 Plant biomass** A higher biomass of a weed holds a negative effect on the neighbor plants in terms of nutrients stolen, the shadow caused and space competition. Data of biomass for each species were recorded over years and have been reported in the MoLTE database in terms of grams of dry matter per species.

**9.1.4.5.3 Plant height** Similarly to the biomass, a taller plant is likely able to catch more sun light than a smaller plant next to it (Craine and Dybzinski 2013). In addition, it likely causes and increase of shadow on the nearby plant. Data of plant height for each species were collected from TRY database.

**9.1.4.5.4 Seed weight** According to Torner et al. (2000), as well as panel experts' opinion, seed weight is sufficient to evaluate seed-related traits for competitiveness as further information might be deduced from seed weight. Indeed, a heavier seed has also more chances to emerge than a lighter seed and a higher seed weight will likely result in a higher plant biomass in the following phenological stages. In addition, a heavier seed has more chances to go deeper into the soil and therefore avoiding external disturbances (such as tillage, machinery passage, run-off etc.) that take place on superficial soil layers, further increasing seed emergence rate. Data of seed weight for each species were collected from TRY database.

**9.1.4.5.5 Drought tolerance** A more drought tolerant plant will be more competitive in a drier soil and hence more competitive in a site featured by extreme environmental conditions such as not-irrigated and dry soils in Mediterranean semi-arid climates. Data of drought tolerance for each species were collected from TRY database.

**9.1.4.5.6 Nitrogen demand** A plant which is well adapted to sites with a low level of nitrogen will be more competitive in soils poor of this element. Nitrogen requirements were evaluated through the Ellenberg Indicator value, ranging between 1 and 9 (Hill et al. 1999). Smaller values (1-3) are associated with plants adapted to N-infertile sites while larger values (7-9) are associated with plant species typical of N-rich sites. Ellenberg data were collected from Ecoflora database (Ecoflora 2022).

**9.1.4.5.7 Shade tolerance** In poor light conditions, plants that are well adapted to shade will be more competitive than species requiring lighter conditions. Shade tolerance was evaluated through the Ellenberg Indicator's Value: smaller values are associated with plant species adapted to shade while larger values correspond to light-lover plants. Ellenberg data were collected from Ecoflora database (Ecoflora 2022).

**9.1.4.5.8 Regulating services** Regulation functions are by far the ones that produce the largest share of ESs and are represented by the largest number of selected FTs (Boerema et al. 2017).

**9.1.4.5.9 Pollination** Pollinators' presence might be affected by herbaceous species growing within the fields as well as by the plant community in the field margins. These species can provide habitat and food for pollinators (Balzan and Moonen 2014; Gabriel and Tscharntke 2007; Gibson et al. 2006; Zhang et al. 2007). Flower morphology is one of the main factors that drives pollinators in flower selection (Fenster et al. 2004). Flower with large perianths, the non-reproductive part of the flower consisting of the calyx and the corolla, triggers high attractiveness to pollinators (Ivey and Carr 2005; Mitchell et al. 2004; Molina-Montenegro and Cavieres 2006). Therefore, Müller classes were used to evaluate the support to pollinators for each weed species. Müller (1883) classified the flowers pollinated by insects into 9 classes, depending on the depth of the nectar source (that is the floral tube length) along with the pollinator proboscis length (Durka 2002). For each weed species found in our survey we gathered information about Müller classification from the BiolFlor database (Version 1.1). The larger the range of typical pollinators associated with a Müller class, the higher was the resulting Müller class score. Müller class scores attributed by an expert entomologist were reported in Appendix C together with Müller class characteristics and corresponding, typical pollinators (online). In addition, flowering period was considered for the ecosystem function of pollination support since longer flowering periods likely result in pollen provisioning over a longer period with a consequent more important service. Flowering period was calculated with BiolFlor data and standardized between 0.0 and 1.0. The final score was calculated as Müller class score weighed by the flowering period value using the following formula:

$$Pollinator\ attractiveness = Müller\ class\ score * 0.7 * Flowering\ period\ score * 0.3 \quad (1)$$

Resulting Pollination scores were grouped in ranges of values from 0.0 to 1.0 (e.g., $0.0 < x < 0.1 = 0.1$, $0.6 < x < 0.7 = 0.7$, etc.).

**9.1.4.5.10 Biological control** In general, there is a direct correlation between the abundance of phytophagous insects and natural enemies. Indeed, it is likely that a higher number of natural enemies, which are carnivorous insects, visits more frequently plants where a wider variety of phytophagous insects feed, regardless whether they are their primary or alternative hosts or preys (Altieri 1999; Price 2011). For each plant species, the number of phytophagous insect species known to feed on it was retrieved from Ecoflora database. The figure of phytophagous insects accounted in the database was expunged from species not recorded for Italy and adjusted on the basis of field surveys conducted in the studied area for years. Moreover, the possible contribution of each plant species as source of non-prey food (nectar, pollen, honeydew) to polyphagous natural enemies was approximately evaluated (Lundgren 2009). On the basis of these overall assessments, a biocontrol supporting score was assigned. The larger the range of herbivorous insect species usually visiting the weed species, and the non-prey food production, the higher is the resulting biocontrol service score ranging from 0.1 to 1. The resulting biological control score was the result of a combination of the number of phytophagous insects retrieved from Ecoflora and the arbitrary considerations of an expert, comparing all the other values from the list. For instance, plants with similar number of visiting phytophagous insects might have different values of biological control score if one attracts only the larvae of the phytophagous and the other one also the adults or depending on the attractiveness of phytophagous (the more attractive for natural enemies, the higher the score).

**9.1.4.5.11 Erosion regulation** For an evaluation of the function of controlling erosion processes, the root architecture, canopy width and the drought tolerance were considered. Root morphology considerably influences soil retention, stabilization and erosion control from run-off processes (Reubens et al. 2007). Anchoring effect of roots depends on their depth and spatial distribution. It has been proved that fibrous and shallower roots are more efficient than tap and deeper roots, respectively, in controlling soil erosion and water regulation (De Baets et al. 2011; Gyssels and Poesen 2003; Zhang et al. 2013). Fibrous roots may potentially control erosion effect 1000 times more than tap roots (De Baets et al. 2007). In addition, a larger coverage of soil, as expressed by canopy width, leads to a lower soil erosion. This phenomenon is crucial especially when extreme climatic events happen (typically in summer) and hence superficial run-off is typically more pronounced. For this reason, also drought tolerance of these species was considered.

**9.1.4.5.12 Water regulation** For studying water regulation service, water infiltration and water storage into the soil were taken into account. Root depth was considered as a FT to evaluate water infiltration. Deeper roots generally lead to a better water infiltration into the soil, as they help to reach deeper soil layers. Leaf dry matter content (LDMC) was considered for evaluating soil water storage capacity, since it is considered as an indicator of soil fertility (Hodgson et al. 2011). Smart et al. (2017) report that LDMC is the best predictor of above-ground net primary production and is a fundamental ecosystem function supporting food production and soil formation. Hence, we assumed for the FunBies model that LDMC is important for evaluating the organic matter that spontaneous species can supply also to soil, improving its structure and therefore increasing water storage capacity. Furthermore, a larger ground coverage reduces the impact of raindrops on the ground and hence lead to higher water infiltration. Data of root depth, LDMC and canopy width were gathered from the TRY database.

**9.1.4.5.13 Climate regulation** Taylor et al. (1989) reported that for substrates low in lignin the C/N ratio is the best predictor of decomposition rate. Although more recent results suggest caution when using certain chemistry ratios to predict decomposition rate in Mediterranean ecosystems, they still confirm C/N correlates negatively with early-stage decomposition rate, which is the most common option in agroecosystems (Bonanomi et al. 2023). It was selected the C/N ratio of leaves – and not the C/N of other parts of the plant – because of the data availability on TRY. This trait gives an idea about the attitude of organic matter of each species to be stocked into the soil and not to be released into the atmosphere (in form of $CO_2$). Instead, small values of leaf C/N ratio reflect a faster decomposition of the plant organic matter with higher rates of $CO_2$ produced. Data of leaf C/N ratio were gathered from the TRY database.

**9.1.4.5.14 Natural hazard regulation** Fire-related plant traits can be used to understand vegetation responses to disturbances from fire regime. In addition, in Mediterranean ecosystems, changes in fire regime might be more relevant than direct changes due to climate changes, making information about fire-related traits crucial (Paula et al. 2009). By fire-related traits we considered traits relevant for plant persistence and regeneration after fire (i.e., post-fire seeding emergence and mortality). Traits information was gathered from the TRY database, which reports on traits ranging between 0.03 and 1.

**9.1.4.5.15 Supporting services** In the supporting service category, cycling of carbon and nitrogen that contribute to soil formation as well as the organic matter decomposition processes of weeds are considered.

**9.1.4.5.16 Soil formation** The carbon present in weed leaves may return into the soil after leaf decomposition. Therefore, an overall higher leaf carbon content results in an increase of carbon amount of the soil as well. Leaf carbon content data were gathered from the TRY database.

**9.1.4.5.17 Nutrient cycling** There are several indices to evaluate the attitude of organic matter to be decomposed into the soil. One of the most common indexes to evaluate it is the leaf C/N ratio, which is available in plant trait databases for most of the herbaceous species. As already mentioned before, the higher the value of plant C/N ratio, the slower the organic matter will be decomposed, the smaller the portion of nitrogen mineralized will be. Similarly to the leaf C/N ratio, the specific leaf area (SLA) index was used to consider the speed of organic matter decomposition. However, in this case, a higher SLA value indicates a thin leaf (large surface/thickness ratio) and hence a fast organic matter decomposition. Data of SLA were gathered from TRY database. N is a fundamental nutrient for plants. However, it needs to be fixed from the atmosphere into the soil to be adsorbed by plant roots. This process requires the symbiosis of roots with N-fixator bacteria. If a plant can establish this symbiosis (typical of leguminous species), a positive coefficient will be assigned to indicate its capability to increase N content of the soil.

**9.1.4.5.18 Cultural services** For this category, we considered the level of importance reached by each species in terms of cultural heritage. The cultural heritage value for each species was calculated as the knowledge score weighted by the use score

$$\textit{Cultural heritage value} = \textit{Knowledge score} * 0.5 + \textit{Use score} * 0.5 \tag{2}$$

The Knowledge score was calculated for each species as the frequency of citations, that is the number of ethnobotanical references where the species was mentioned over the total. To this purpose, we selected a list of ethnobotanical references concerning the traditional knowledge of plants in Tuscany region (Camangi et al. 2007; Corsi and Pagni 1979; Frassinelli 2008; Molines 2018; Randellini 2007; Signorini et al. 2007). The higher the frequency of citations, the higher the knowledge score: if a species is cited in all the six references considered, the score is the highest, if it is never cited is the lowest. Similarly to the knowledge score, we calculated the Use score of each species depending on its number of traditional uses reported in the considered bibliographic references. We took into account the following uses: cosmetic, craft, domestic, dyer, food, liquor use, magical, medicinal, ornamental, recreational, religious and veterinary. As the knowledge score, the higher the uses, the higher the resulting score. Finally, the cultural heritage values were grouped in ranges of values from 0.1 to 1, i.e. $0.0 < x < 0.2 = 0.1, 0.2 < x < 0.4 = 0.3, 0.4 < x < 0.6 = 0.5, 0.6 < x < 0.8 = 0.7, 0.8 < x < 1 = 0.9$.

#### 9.1.4.6 Integrated assessment of functional biodiversity

**9.1.4.6.1 Aggregation of ecosystem services provided by plant functional traits** Integrated assessment of functional biodiversity within the FunBies model was implemented by constructing a specific LAM model to aggregate species/trait performances at the level of ES category and for calculation of one overall functional biodiversity index (FBI).

A linear additive multi-criteria model is commonly used to combine many indicators into one overall value (Dodgson et al. 2009). It allows reducing information from many individual indicators into a single summarized index, easier to interpret and more accessible to decision makers and public. The linear additive structure of aggregation allows to give different importance to the elements composing the model: the value score on each element (FT in our model) is multiplied by the weight assigned to that element (Paracchini et al. 2011). After, the weighted scores of all indicators will be summed up to give the contribution of a given species for a number of ecosystem functions within each of the provisioning, supporting, regulating and cultural ES categories. The values obtained in such a fashion will be further weighted at the level of ES category and then summed together to obtain one overall value for each of the species of a crop weed community. If we sum up the species values we obtain an overall value, i.e. the functional biodiversity index of a given crop-weed community, as shown in the following equation:

$$FBI = \sum_{Sp=1}^{N} \sum_{ES=1}^{4} w_{ES} * \left[ \sum_{EF=1}^{x} w_{EF} * \left( \sum_{FT=1}^{n} w_{FT} * A_{Sp} * S_{FT} \right) \right] \quad (3)$$

Where $W_{ES}$ is the weight attributed to each of the four ecosystem service categories, $W_{EF}$ is the weight attributed to each ecosystem function, $W_{FT}$ is the weight attributed to each functional trait, $A_{Sp}$ is the abundance of a species either in terms of number of individuals (nr/m$^2$) or of dry matter weight (g/m$^2$) $S_{FT}$ is the FT score per each species unit expressed either in terms of number of individuals or grams.

One of the requirements for processing multiple indicators within an aggregation framework is that all are reduced to the same scale, with common units (Nardo and Saisana 2005). Thus all indicators must be standardized, preferably to a continuous numerical scale, in order to allow mathematical procedures such as linear-additive aggregation to be performed (Paracchini et al. 2011). FT scores representing the potential ability of a plant to provide a given ecosystem service, or cause a disservice, vary between 0 and 1 and were standardized based on FT-specific ranges of values.

Standardisation was carried out in such a manner that scores close to one represent higher benefits and scores negatively weighted represent disservices. Specific ranges are reported in Figure S33, with relevant measure units, under corresponding FTs. The range within which FT values are standardized should include potential FT values for a large number of species and in some cases could be truncated to omit too high or too low values of outliers that would cause underestimation of differences between all other species. Seed weight values, originally ranging between 0.05 and 1531g, were log-transformed, which reduced the range between 0.05 and 310g.

If we suppose $A_{Sp}$=1, by sequentially aggregating FT scores at the levels of EFs and ES categories we obtain a functional biodiversity index (FBI) at species level that ranges between 0 and 1. It has to be noticed that this specific FBI represents the contribution that each species single unit can supply to functional biodiversity. Of course, the more abundant is a species in a field, in a hectare or in whatever reference area, the more it can contribute to overall functional biodiversity. In the present case species abundance was measured by both number of individuals (nr/$m^2$) and dry matter weight (g/$m^2$). Each weighted FT score was multiplied either by dry matter weight or by number of individuals depending on which of these two measure units would fit better the selected FT indicator. Coherently each FT score was either referred to a single unit of number of individuals or of dry matter weight.

An example of calculation procedure for functional biodiversity index at single species level is given in Appendix D and Table S38.

By summing FBIs calculated for each single species belonging to crop-weed communities we obtain an overall FBI that can represent functional biodiversity performances at the level of OO, NO or CO micro- agroecosystem, or whatever else assemblage of species in a given agroecosystem.

**9.1.4.6.2 Expert validation** The conceptual and the LAM models including plant FTs, FT scores and weights were validated by a panel of experts. Noble (2004) defined a panel of experts as a "group of informed individuals selected to assign impact assessment judgment based on experience and expertise". Indeed, expert-based assessment is the most appropriate approach to validate indicators when no real, quantitative data based on observations are available (Paracchini et al. 2011). The panel was composed by members with different expertise so that they could validate coefficients of a wide range of ESs. In addition, gathering together experts with different scientific backgrounds ensured interactions and discussions leading to a reinforced validation.

The "Expert Panels" guidelines proposed by the JRC of European Commission (Torner et al. 2000) were followed to establish the size and composition of the panel, gathering members together and choosing a panelist chair. Following, the step-by-step guide was implemented to carry out the procedure for validation. First, the size of the panel was decided depending on the objective of the impact assessment and the available time and resources. The composition of the panel was based on criteria withdrawn from Noble (2004). Criteria were as follows:

- Experience: i) knowledge of two or more of the specialty areas considered in the assessment, ii) 7-10 years of combined education and professional experience in impact assessment;
- Reputation: i) publications, ii) participation in professional meeting and/or symposia, iii) panelist's involvement in similar types of projects, iv) appropriate geographic representation;

- Heterogeneity of the panel.

A first call was sent them on the 7th of August 2017 with the description of the project including detailed background information along with the request of taking part in the panel. Finally, a panel composed by 9 experts was established, which is presented in Table S27. At this stage we gave preference to academic experts. Indeed, expert validation did not focus only on the aggregation procedure including FT scores and weights; also the overall architecture of the FunBies was scrutinized, which comprises an empirical-statistical, crop-weed community component, a trait-based conceptual model, and a linear additive multi-criteria (LAM) model. FunBies was constructed based on multi-faced scientific knowledge from MVA statistics, functional ecology, economics and mathematics, which requested, besides scientific background on single agroecosystem components and processes, a more general expertise on scientific research methods.

Information regarding background of the panelists, including previous experiences, publications, meetings and other panel contributions was collected from each member (Table S28).

Once the panelist chair was chosen, scoring systems and weights for each FT were identified by the authors based on the literature review. Then, the procedure for validation was implemented, which consisted in two phases. First, a one-to-one meeting with the panel chair and each panelist was organized. In this meeting, the panel chair presented and discussed the overall FunBies multi-criteria framework and assessed together with each expert corresponding FT scores and weights. Second, a plenary meeting was organized on the 13th of October 2017 to discuss and officially validate FT selection and corresponding scores and weights. In the course of the plenary session each FT scoring system and weight was submitted to the whole panel of experts in order to ensure a truly inter-disciplinary validation of the LAM model. Furthermore, standardization rules of FT scores were established and assessed.

### 9.1.5 Results

**9.1.5.1 Selection of most representative crop-weed communities** In Figures S27, S28, S29, S30, S31, S32 cluster dendrograms and MDS plots ordering sample observations of crop-weed communities of OO, NO and CO micro-agroecosystems collected at MoLTE in the period 1993-2017 are reported, respectively. Observations were ordered with the aim to model the provision of ecosystems services based on the most representative crop categories of the reference area. MVA representations proved to be reliable and useful in the FunBies model construction, considering the extreme diversity of sample individuals. Stress values of MDS plots lie between 0.19 and 0.21. According to Clarke and Warwick (2001), a stress value between 0.1 and 0.2 gives a potentially useful 2-dimensional picture, though for values at the upper end of the range a cross-check of the groupings should be made by superimposing CA groups of farms. OO, NO and CO crop-weed communities were ordered in two major groups at a within-group similarity level of 10% (OO and CO) and 15% (NO). In all of the three micro-agroecosystems the two groups represented homogeneous crop categories, i.e. Group 1 (labelled with a star in Figures S28, S30 and S32), including mainly WCs and LFs, and Group 2 (labelled with a triangle), including mainly RCs and LGs. The only exception to this pattern was due to the absence of legume crops in the CO micro-agroecosystem.

In Figures S28, S30 and S32 clusters were superimposed on MDS plots. While the level of determination of membership of each sample to one of the two groups was made possible at higher detail thanks to the superimposition of clusters, inter-relations between the samples on a continuous scale were displayed thanks to the MDS configuration on the plot. Clusters are not imposed because the continuum of change remains visible on corresponding MDS plots. Some sample individuals were positioned in the overlapping space between two different groupings when MDS and CA were combined: their attribution to groups was ambiguous. Allocating each sample to a single group (including those in the intersections) was made possible by checking their single membership on the CA dendrogram.

Regarding OO (Figure S28), exceptionally, 04BAR17 belonged to the RC group, as weed species usually found within RCs were collected in this barley field. 01BAR05 and 01CLOVER08 sample individuals were considered outliers since they resulted as a separate group. In addition, by superimposing clusters at a degree of similarity of 45% on the MDS plot we isolated groups characterized by LF and LG crop-weed communities that were embedded in larger C and RC groups, respectively.

Concerning NO (Figure S30), eight groups of samples were identified at a degree of similarity of

10%. Two overlapping groups were composed by WC and LF crop-weed communities and were merged (Group 1). Similarly, three groups were characterized by RC and LG communities and were merged as well (Group 2). Other groups, resembling in total only four sample individuals (i.e. 07CLOVER08, 08LUCERNE12, 06MAIS03 and 08BAR04) were considered as outliers. Regarding CO (Figure S32), two groups were identified at 10% of similarity. One is characterized by RC communities, while the other group is mainly featured by WC communities. Overall, we identified throughout all of the three OO, NO and CO micro-agroecosystems two macro-groups of crop-weed communities, i.e. WC+LF and RC+LG, which were later characterized in terms of community composition and contribution of the most representative species to within-group similarity.

**9.1.5.2 Characterization of the most representative crop-weed communities** In Table S29 the plant species which contribute the most to the within-group similarities of each of the WC+LF and RC+LG macro-groups of crop weed communities are reported for each OO, NO and CO agro-ecosystem management option.

Results of SIMPER analysis show that in general selected crop weed communities are featured by low levels of within-group similarity, ranging from 16.9% in the OO RC+LG group to 22.2% in the OO WC+LF group. Notwithstanding this aspect, which is in line with high levels of biodiversity found in the area, groups of crop weed communities were identified in an unambiguous way and were consolidated by SIMPER results in terms of group composition. Average species richness of crop-weed-communities per macro-group category in the period 1993-2017 slightly changed from 13-14 species in OO, to 12-14 species in NO and12 species in CO micro-agroecosystems. If we cut-off from the total number of species those that contribute the least to within-group similarity, i.e. those species that cumulatively account for 10% or less of within-group similarity, we found that OO and NO showed higher variety of representative species as compared to CO crop-weed communities both for WC+LF crops (i.e. 9, 9 and 7 species, respectively) and for RC+LG crops (14, 10 and 7 species, respectively).

In all of the groups species that mostly contribute to within-group similarity are those that in the course of 25 years have been stably present. Often, with very few exceptions (e.g. *Trifolium pratense* L. in OO WC+LF group), those species also held higher average abundances and can be considered dominant in corresponding weed communities.

Among those species that mostly contributed (more than 10%) to within-group similarity *Fallopia convolvulus* L. and *Polygonum aviculare* L. characterized WC+LF communities of both OO, NO and CO micro-agroecosystems (33.7-23.9, 26.8-13.3 and 25.2-26.7 %, respectively).

*Convolvulus arvensis* L. characterized WC+LF communities of both NO and CO (14.1 and 14.5%, respectively) and, to a minor extent, of OO (4.9%). There was not difference between organic and conventional systems regarding dominant species in WC+LF communities. It seems that competition power of WC+LF crops is high and few species can withstand it. However, if we consider additional representative species (those that cumulatively represent 90% or more of within-group similarity, excluded the already mentioned dominant species), these systems differ to a broad extent. Four of 6 additional, representative species of OO are equal to those of NO. CO holds only 1 of 4 additional species that is equal to those of OO or NO.

Concerning RC+LG crops, we found even broader difference between organic and conventional crop-weed communities.*Setaria italica* L. *P.Beauv.* subsp. *viridis* L. was found to be the dominant species for OO and NO communities (18.4 and 34.2 %, respectively), followed by *Sinapis arvensis* L. and *Sorghum halepense* L. in OO communities (13.2 and 11.9%, respectively) and by *Sonchus asper* L. in NO systems (19.2%). In CO communities *Convolvulus arvensis* L., *Cirsium arvense* L. and *Sorghum halepense* L. resulted to be the most representative species (37.3%, 19.4 and 11.8%, respectively). Nine of the 10 most representative species of NO communities are included in the 14 most representative OO species, while this applied to only 3 of the 7 most representative CO species.

Overall, there appears to be a remarkable difference between community composition of organic and conventional WC+LF crops, although potential impact on functional biodiversity by dominant species could be similar. Instead, organic and conventional communities of RC+LG crops seem to be broadly different, which should give rise to corresponding differences in terms of impacts on bio-functionality.

**9.1.5.3 Results of the FunBies model** FunBies can supply a broad range of results in terms of services produced by a single FT, by a single EF, by aggregated groups of EFs (i.e., provisioning, supporting, regulating and cultural), or of an overall functional biodiversity index. Besides, these results can refer both at the contribution of a single species to functional biodiversity or of an entire plant community. As an example of how FunBies can generate useful outcomes for integrated assessment of functional biodiversity in the following we will present results of the overall functional biodiversity index at system level, of FBI per crop macro-group (WC+LF and RC+LG, respectively) and at species level.

**9.1.5.4 Results of the overall functional biodiversity index at system level** In Figure S34 FBI results at the level of OO, NO and CO systems are presented, respectively, under two different scenarios: equal weight scenario (WS) and expert-based WS. In the equal weight scenario each ES category holds the same weight, i.e. 0.25, while as an alternative experts proposed weights as follows: 0.5 for the provisioning category, 0.2 for the regulating and supporting categories and 0.1 for the cultural category. In this way experts acknowledged the widespread perceptions that weeds are mainly elements of competition against crops and that cultural aspects are secondary.

Results of FBI under the two scenarios did not differ in relative terms. OO showed the best performance (19.32 and -31.03 under the equal and expert-based WSs, respectively) and CO the worst (5.78 and -54.02, respectively), with NO laying in between (13.16 and -35.23, respectively). NO and CO produced 32% and 70% less overall ESs than 00 under the equal WS, respectively, and showed a 14% and 74% lower FBI under the expert-based WS, respectively. It seems that organic management outperforms conventional for what concerns functional biodiversity and that this difference increases in more mature systems; indeed, OO was converted to organic production 10 years before NO. These differences only slightly modified under different WS.

**9.1.5.5 Provision of ecosystem services per macro-group of crop-weed communities** In Figures S35 and S36 provision of ecosystem services by representative WC+LF and RC+LG crop-weed communities in OO, NO and CO micro-agroecosystem at MOLTE is presented. In this figure we decided to show results by single EFs in order to interpret at a more detailed level the results of the overall functional biodiversity index. EFs considered were erosion regulation, water regulation, pollination, biocontrol, climate regulation and natural hazard regulation (for regulating services), cultural heritage (cultural service), soil formation and nutrient cycling (supporting services) and competitiveness (provisioning service).

Concerning WC+LF, it is evident that OO performed better than NO, which in turn performed better than CO. This is in line with the results of the overall FBI previously shown. Specifically, the spider diagram shows how OO achieved the highest performance regarding erosion and water regulation, pollination, biological control and cultural heritage.

Unexpectedly, results revert when we consider RC+LG crop category. In this case CO performances were higher especially for what concerns climate regulation, supporting services and competitiveness. This can be explained by the large importance that Convolvulus arvensis holds within the CO RC+LG crop-weed communities (Table S29, 37.3% of within-group similarity contribution) combined with overall second-best performance of this species in terms of regulating and fifth-best for provisioning dis-service (Figure S37 and Appendix D, Table S37, scores of 0.83 and 0.19, respectively).

Concerning the impact of these EFs on the FBI of RC+LG crop-weed communities, it has to be noticed that the beneficial effects of supporting services and climate regulation are partially counterbalanced by the negative impact due to competitiveness.

**9.1.5.6 Results of the functional biodiversity index at species level** In Figure S37 results of the application of FunBies at speciel level are reported, which are specified in Appendix D. Most competitive species resulted to be *Helianthus tuberosus* L., *Helianthus annuus* L. and *Sorghum halepense* L. (provisioning scores equal to -0.35, -0.33 and -0.26, respectively), followed by *Medicago sativa* L. and *Convolvulus arvensis* L. (0.20 and 0.19, respectively). It has to be noticed that both *Helianthus annuus* L. and *Medicago sativa* L. are ordinary crops used in the rotations and are mainly present as residual individuals of preceding crops. Best performing species for regulating services are *Cirsium arvense* L., *Convolvulus arvensis* L. and *Dactylis glomerata* L. (1.00, 0.83 and 0.44, respectively), for supporting services are *Medicago lupulina* L., *Trifolium pratense* L. and *Veronica persica* Poir. (0.78, 0.76 and 0.70, respectively), for cultural services are *Papaver roheas* L., *Equisetum arvensis* L. and *Daucus carota* L. (1.00, 0.62 and 0.40, respectively.

### 9.1.6 Discussion and Conclusions

The objectives of the present research were to describe FunBies model, to show how it was validated and to present results of its application for the quantification of ESs delivered by weed communities of organic vs conventional systems. In this section we will discuss validity and validation processes of FunBies single components and results of its application.

**9.1.6.1 Valuation of the FunBies crop-weed community component** To our knowledge no model was developed able to predict the evolution of a vegetation community in cultivated fields under the disturbance imposed by different management techniques on site scale. It is common for agronomists to model the impact of weeds on a given crop but not vice versa. This aspect must not be underestimated if we want to model the contribution of weed communities to ESs produced in agroecosystems. Ecologists seem to be one step forward in this direction: You et al. (2015) carried out a review of ecological models of riparian vegetation under disturbances. Outcomes of the review are particularly important as riparian vegetation communities hold similarities with vegetation communities in cultivated fields, i.e. crop-weed communities, in terms of the quantity and, to a given extent, quality of anthropogenic and climate disturbances they suffer. They identify three types of models commonly used in the study of vegetation communities: statistics-based, empirics-based and analytics-based.

The crop-weed community component in FunBies is indeed designed as an empirical model. A general empirical model is based on field data, experiments, natural rules of the environment, and vegetation attributes such as biomass, density or richness of species, whereas the features of the experimental method are reasonable assumption and accurate control on setting sample plots, controlling the experimental progress, and explaining the result or phenomena (You et al. 2015).

The FunBies crop-weed component was built based on a 25-year-old database that includes 223 records on biomass, density and richness of species collected within organic and conventional fields of the Montepaldi long term experiment. They cover 97.6% and 70.4% of crop categories and crop species, respectively, as indicated by the last Italian census of agriculture for Tuscany inland hill arable land (Istituto Nazionale di Statistica (ISTAT) 2010). Crop-weed community samples were collected in the same experimental site (i.e., MOLTE) to allow for comparison between alternative cropping systems under the same soil conditions. Rotations slightly changed concerning crop species during 25 years due to climate change (sunflower replaced maize) and market reasons (LG partially replaced LF), which resulted in a broad range of crops sampled under different climatic conditions.

Besides, the empirical model was refined using MVA statistics. Such a wealth of observations was ordinated according to similarity among communities of crop categories and corresponding virtual, representative weed communities for both organic and conventional rotations typical of Tuscany inland hill arable land were modelled considering average species richness and species mostly contributing to within-group similarity.

**9.1.6.2 Valuation of the FunBies conceptual model: a trait-based approach** Zakharova et al. (2019) reviewed two decades of trait-based modelling in ecology. They state that trait-based models often require less parameterization effort than species-based models, facilitate scaling-up, and produce more generalizable results that can be projected to other systems, which is a highly appreciable feature in applied ecology studies. Furthermore, trait-based modelling reinforces simplification, which is at the core of all modelling. They see potential for the reinforcement of trait-based modelling approaches in areas such as the assessment of ecosystem services, biodiversity studies and, especially, the prediction of community and ecosystem responses under climate and land-use changes.

However, even the most recent studies dealing with trait-based models of ecosystem services developed for the agricultural sector focus only on grassland management in semi-natural habitats (Lochon et al. 2018; Schirpke et al. 2017), with none considering arable cropping systems. Furthermore, they privilege the depth of the modelling approach used to assess land-use option performances at the expense of the wideness of ESs considered ("only" five, i.e. forage production and quality, soil fertility, water quality and carbon storage).

FunBies conceptual model consider all of the MA ES categories and 10 different EFs that cover all EFs of De Groot's classification (De Groot et al. 2002) among those ascribable to weed communities in agroecosystems, i.e. climate regulation, disturbance prevention, water regulation, soil retention, soil formation, nutrient regulation, pollination, biological control, competition towards production functions of food, raw materials, genetic, medicinal or ornamental resources, cultural and historic information. Besides, FTs considered for aggregated assessment of functional biodiversity in FunBies relate to the whole set of plant organs including leaves but also stem and roots, which is in line with the plant economics spectrum approach to ecosystem service provision (Reich 2014).

**9.1.6.3 Validation of the FunBies linear additive multi-criteria model** All elements of the above reported aggregation scheme, including the standardization procedures, the three weighting systems and the FT ranges were assessed using a face validity test carried out by an independent panel of experts. Testing for face validity was chosen as the validation procedure as it is the most appropriate approach when no real-system data are available (Qureshi et al. 1999).

Aggregation in FunBies of FT indicators is based on a LAM model. In general, as reported from Dodgson et al. (2009) “Models of this type have a well-established record of providing robust and effective support to decision makers working on a range of problems and in various circumstances”. However, this flexibility is subject to the condition that the assessment criteria (represented by FT indicators in the present scheme) are mutually preference independent. Mutual independence of preferences is obtained by imposing to indicators FT ranges so that preference of any given criterion is unaffected by preference on the others (Dodgson et al. 2009). In this way we achieved a conceptually and theoretically robust structure of the FunBies FT indicator aggregation scheme (Fig. S33).

**9.1.6.4 Example of application: organic vs conventional** FunBies was applied to compare organic vs. conventional management options and supplied outcomes at different levels including the overall FBI calculated at cropping system level (OO, NO and CO Fig. S34), FBI calculated at crop category level (WC+LF and RC+LG, Figs. S35 and S36, respectively) and FBI calculated at species level (Appendix D, Table S37).

Results at cropping system level clearly indicated that organic systems have the potential to supply considerably higher ESs than conventional systems, where chemical-synthetic herbicides and fertilizers were applied (Appendix A, Table S30). Demand of ecosystem services is increasing worldwide as well as knowledge of which agro-ecosystem management option can best host EFs providing them. FunBies was developed to answer this demand of knowledge and, at least for the present application, seem to be able to do it. Even more interestingly, FunBies could capture the dynamics of ES provision in time. Indeed, looking at the overall FBI outcomes in Figure S34, it is clear that there is a steady increase of ES provision starting from time of conversion from conventional to organic management. It seems that the ES provision increases together with the evolution of the phytocoenosis.This particular aspect is confirmed at the level of WC+LF crops (Fig. S35), even accompanied by a considerable diversification of ESs, especially towards regulating and supporting services. Acquiring knowledge on these aspects is of vital importance in view of improved understanding of the complex dynamics underlying ecosystem service provision, which involve multiple trophic levels including e.g. insects responsible for pollination and biocontrol or micro-organisms responsible for nutrient cycling and nutrient formation. As stated by Lavorel et al. (2009) trait linkages within and across trophic levels can also guide ecological engineering through the choice of plant trait assemblages that promote the recovery of a multi-trophic community most likely to provide the desired ecosystem services. FunBies has the potential to help in such an intervention as single plant species fitness to hold trophic relations geared to the above-mentioned ESs can be easily verified by withdrawing relevant information on ESs at species level(Fig. S37 and Appendix C, Table S36).

Agroecosystems dynamics are overwhelmingly complex and, indeed, results of ESs for RC+LG crops are reverted as compared to WC+LG(Figs. S36 and S35, respectively), with the only exception of pollination. Higher CO performances in terms of nutrient cycling, soil formation and climate regulation are partially counterbalanced by the competitiveness negative impact. All of these services are related to carbon and nutrient cycling processes, which are primarily driven by traits of the most abundant (dominant) species according to "the biomass ratio hypothesis" by Grime (1998). In CO RC+LG crop category the dominant species in *Convolvulus arvensis* L., i.e. one of the best performing species for the above-mentioned ESs. In this context and for the relevant ESs, FunBies seems to be in line with Grime's hypothesis.

However, what FunBies is not able to do is to assess niche complementarity that might result by non-overlapping trait distributions for some of the other EFs. In OO and NO RC+LG weed communities species evenness is considerably higher as resulted from MVA statistics (Table S29, 14 and 10 species cover 90% of contribution of within-group similarity in OO and NO RC + LG, respectively, versus only 7 in CO RC) and this could have a positive effect in terms of such functional complementarity. E.g., Woodcock et al. (2019) published a meta-analysis revealing how management practices increasing not just pollinator abundance, but also functional divergence, could benefit oilseed rape agriculture, and this could be also applied to functional divergence of those plants that host pollinators and therefore indirectly increase the pollination service.

Another feature that is not supported by FunBies, which could cause underestimation of OO and NO ESs is that intra-specific variability is not considered. All individuals of a species are considered equal in terms of the level of ESs they supply, regardless if they grew in an organic or a conventional field, while it is reasonable to think that use of herbicides could depress relevant EFs.

In the present exercise FunBies was applied at cropping system level to compare organic and conventional agriculture; however, it could be easily adopted for alternative phytocoenosis databases, including those of farm semi-natural habitats and ecological infrastructures. FunBies empirical database offered a wealth of data on floristic richness under a 25-year long time-span featured by changing climatic conditions and a vast range of crops. Although pedo-climatic conditions of MoLTE can be considered to a given extent as representative of Tuscany inland hill arable land, the extent to which this assumption applies is questionable.

For instance, FunBies was not calibrated and tested in ordinary farms, where management conditions in terms of timing of operations, care and control and expert knowledge available can differ from those of an experimental context. As for all models, the extent to which FunBies can be considered applicable depends on the specific aim and the scope of the application, which could result in limitations in the use of this model. Indeed, FunBies calibration and testing in ordinary farms is a further step of the present research process.

### 9.1.7 Concluding remarks

FunBies was validated and tested and showed strong potential to assess ES performance of weed communities at production system, crop and species levels and at different levels of aggregation. Its validity is confined to Tuscany inland hill arable land, which is the reference area of MOLTE experimental fields, rotation and crops, where we expect to find very similar crop-weed communities. The extent to which these expectations are acceptable depends on the specific aim of the proposed application and further testing and calibrations in ordinary farms. Provided that region-specific testing and calibration were performed, FunBies (more specifically its conceptual and aggregation components) hold the potential to be applied in several agroecological contexts, paving the way to a new, critical, and scientific way to evaluate weed ecosystem services.

The FunBies application showed in the present article give hints on how this tool could be used under a number of different contexts. Among them we see two of major importance: (i) design of biodiversity components within agro-ecosystems to optimize ES provision, and (ii) justification and sizing of organic and more in general agri-environmental payments of rural development plants. Concerning this last point, the way in which FunBies is formulated would facilitate integration with any kind of integrated ecological-economic farming systems model and matching of ES provision figures with figures retrieved from ecological models on e.g. potential risk of pesticide use, nitrogen leaching and soil erosion.

### 9.1.8 Tables

Table S27: Panel of experts selected for validation of the FunBiES model. For each ecosystem service (ES) category, the corresponding functional traits (FTs) are shown together with required expertise and selected experts.

| Ecosystem service category | Functional trait(s) | Expertise | Name of expert(s) |
|---|---|---|---|
| Provisioning | Weeds and competition | Weed scientist, Ecologist | Argenti, Vazzana |
| Regulating | Roots, water/climate regulation and soil retention | Agronomist, Pedologist | Napoli, Certini |
| | Pollination and biocontrol | Entomologist | Sacchetti |
| Supporting | Soil formation and nutrient cycling | Soil scientist, Botanist | Ceccherini, Bussotti |
| Cultural | Cultural heritage and local memory of the use | Botanists | Selvi, Viciani |

Table S28: Information about experts' background. Each capital letter in the columns is referred to a member of the panel.

| Experts background | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
| Total years of practice/experience[1] | 25 | 25 | 12 | 20 | 12 | 20 | 25 | 30 | 20 |
| Number of publications on the topic[1] | 20 | 20 | 10 | 10-15 | 12 | 10 | 20 | 100 | 15 |
| Presentations at conventions[1] | 5 | 5 | 1 | 2-3 | 2 | 1 | 5 | 50 | 2 |
| Holds/held leadership/management positions in ES assessment | No | No | No | No | No | No | No | Yes | No |
| Currently active in the area of ES assessment | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No | Yes |

Legend: A, Prof. G. Argenti; B, Prof. F. Bussotti; C, Dr. M.T. Ceccherini; D, Prof. G. Certini; E, Dr. M. Napoli; F, Prof. P. Sacchetti; G, Prof. F. Selvi; H, Prof. C. Vazzana; I, Prof. D. Viciani;

[a]Related to agronomical-environmental subjects.

Table S29: Results of similarity percentage (SIMPER) analysis for crop-weed communities of old organic (OO), new organic (NO) and conventional (CO) macro-groups of crops found at Montepaldi long term experiment, San Casciano Valdipesa, Florence, Tuscany. Macro-groups of crops are winter cereals (WC) plus legumes for forage (LF), and raw crops (RC) plus legumes for grain (LG). Macro-group average similarities: Old Organic – WC+LF = 22.2; Old Organic – RC + LG = 16.9; New Organic – WC+LF = 19.5; New Organic – RC + LG = 20.4; Conventional – WC+LF = 19.0; Conventional – RC + LG = 21.3).

| Species | Average abundance | Average similarity | Contribution to group similarity (%) | Cumulative contribution (%) |
|---|---|---|---|---|
| Old organic – WC+LF (n=13) | | | | |
| *Fallopia convolvulus* (L.) Á.Löve | 23.3 | 7.5 | 33.7 | 33.7 |
| *Polygonum aviculare* L. subsp. *Aviculare* | 22.8 | 5.3 | 23.9 | 57.6 |
| *Lolium multiflorum* Lam. | 37.2 | 2.1 | 9.5 | 67.1 |
| *Convolvulus arvensis* L. | 5.8 | 1.1 | 4.9 | 72.0 |
| *Anthemis arvensis* L. | 11.1 | 1.0 | 4.6 | 76.6 |
| *Stachys annua* L. subsp. *annua* | 5.8 | 0.9 | 4.1 | 80.7 |
| *Lysimachia arvensis* (L.) U.Manns & Anderb. | 5.2 | 0.9 | 4.0 | 84.7 |
| *Sinapis arvensis* L. subsp. *arvensis* | 5.1 | 0.7 | 3.1 | 87.9 |
| *Trifolium pratense* L. | 50.0 | 0.7 | 3.0 | 90.9 |
| *Kickxia spuria*(L.) Dumort. | 6.5 | 0.4 | 1.9 | 92.8 |

| Species | Average abundance | Average similarity | Contribution to group similarity (%) | Cumulative contribution (%) |
|---|---|---|---|---|
| *Euphorbia helioscopia* L. subsp. *helioscopia* | 2.5 | 0.3 | 1.6 | 94.3 |
| *Papaver rhoeas* L. subsp. *rhoeas* | 1.0 | 0.2 | 1.0 | 95.3 |
| *Lolium perenne* L. | 2.8 | 0.2 | 0.7 | 96.0 |
| Old organic – RC + LG (n=14) | | | | |
| *Setaria italica* (L.) P.Beauv. subsp. *viridis* (L.) Thell. | 9.1 | 3.1 | 18.4 | 18.4 |
| *Sinapis arvensis* L. subsp. *arvensis* | 3.1 | 2.2 | 13.2 | 31.6 |
| *Sorghum halepense* (L.) Pers. | 3.1 | 2.0 | 11.9 | 43.5 |
| *Fallopia convolvulus* (L.) Á.Löve | 1.5 | 1.6 | 9.7 | 53.2 |
| *Convolvulus arvensis* L. | 1.6 | 0.9 | 5.2 | 58.4 |
| *Stachys annua* L. subsp. *annua* | 0.7 | 0.8 | 4.4 | 62.8 |
| *Anthemis arvensis* L. | 1.6 | 0.7 | 4.4 | 67.2 |
| *Helminthotheca echioides* (L.) Holubs | 2.6 | 0.7 | 4.1 | 71.3 |
| *Sonchus asper* (L.) Hill | 1.8 | 0.7 | 3.9 | 75.2 |
| *Kickxia spuria* (L.) Dumort. | 1.1 | 0.6 | 3.5 | 78.7 |
| *Lysimachia arvensis* (L.) U.Manns & Anderb | 0.8 | 0.6 | 3.4 | 82.1 |
| *Cynodon dactylon* (L.) Pers. | 1.6 | 0.5 | 3.1 | 85.2 |

| Species | Average abundance | Average similarity | Contribution to group similarity (%) | Cumulative contribution (%) |
|---|---|---|---|---|
| *Lolium perenne* L. | 1.2 | 0.5 | 2.9 | 88.0 |
| *Chenopodium album* L. | 1.1 | 0.4 | 2.3 | 90.3 |
| New organic – WC+LF (n=12) | | | | |
| *Fallopia convolvulus* (L.) Á.Löve | 27.8 | 5.2 | 26.8 | 26.8 |
| *Lolium multiflorum* Lam. | 56.0 | 3.6 | 18.6 | 45.4 |
| *Convolvulus arvensis* L. | 9.1 | 2.7 | 14.1 | 59.4 |
| *Polygonum aviculare* L. subsp. *aviculare* | 15.3 | 2.6 | 13.3 | 72.7 |
| *Anthemis arvensis* L. | 12.5 | 1.1 | 5.5 | 78.2 |
| *Sinapis arvensis* L. subsp. *arvensis* | 3.4 | 0.8 | 4.3 | 82.6 |
| *Stachys annua* L. subsp. *annua* | 6.6 | 0.8 | 4.0 | 86.5 |
| *Lolium perenne* L. | 11.3 | 0.6 | 2.8 | 89.4 |
| *Galium aparine* L. | 3.9 | 0.3 | 1.7 | 91.1 |
| *Euphorbia helioscopia* L. subsp. *helioscopia* | 3.1 | 0.3 | 1.6 | 92.7 |
| *Fumaria officinalis* L. | 1.4 | 0.3 | 1.5 | 94.2 |
| *Lysimachia arvensis* (L.) U.Manns & Anderb | 2.1 | 0.2 | 1.1 | 95.3 |
| New organic – RC + LG (n=14) | | | | |

| Species | Average abundance | Average similarity | Contribution to group similarity (%) | Cumulative contribution (%) |
|---|---|---|---|---|
| *Setaria italica* (L.) P.Beauv. subsp. *viridis* (L.) Thell. | 21.6 | 7.0 | 34.2 | 34.2 |
| *Sonchus asper* (L.) Hill | 12.4 | 3.9 | 19.2 | 53.4 |
| *Fallopia convolvulus* (L.) Á.Löve | 1.5 | 1.3 | 6.3 | 59.7 |
| *Setaria verticillata* (L.) P.Beauv. | 9.7 | 1.2 | 5.8 | 65.5 |
| *Sinapis arvensis* L. subsp. *arvensis* | 3.9 | 1.1 | 5.4 | 70.9 |
| *Sorghum halepense* (L.) Pers. | 2.1 | 1.0 | 5.0 | 75.9 |
| *Stachys annua* L. subsp. *annua* | 1.8 | 1.0 | 4.8 | 80.7 |
| *Euphorbia prostrata* Aiton | 1.9 | 0.8 | 4.0 | 84.6 |
| *Convolvulus arvensis* L. | 2.1 | 0.8 | 3.8 | 88.4 |
| *Chenopodium album* L. | 1.6 | 0.7 | 3.5 | 92.0 |
| *Anthemis arvensis* L. | 3.8 | 0.5 | 2.4 | 94.3 |
| *Kickxia spuria* (L.) Dumort. | 1.1 | 0.3 | 1.7 | 96.0 |
| *Lolium perenne* L. | 0.8 | 0.3 | 1.2 | 97.2 |
| *Cirsium arvense* (L.) Scop. | 1.1 | 0.2 | 0.8 | 98.0 |
| Conventional – WC+LF (n=12) | | | | |
| *Polygonum aviculare* L. subsp. *aviculare* | 22.2 | 5.1 | 26.7 | 26.7 |
| *Fallopia convolvulus* (L.) Á.Löve | 20.4 | 4.8 | 25.2 | 51.9 |

| Species | Average abundance | Average similarity | Contribution to group similarity (%) | Cumulative contribution (%) |
|---|---|---|---|---|
| *Convolvulus arvensis* L. | 5.0 | 2.8 | 14.5 | 66.5 |
| *Lysimachia arvensis* (L.) U.Manns & Anderb | 21.9 | 1.7 | 8.9 | 75.3 |
| *Galium aparine* L. | 7.3 | 1.5 | 7.7 | 83.0 |
| *Veronica persica* Poir. | 9.0 | 0.7 | 3.7 | 86.8 |
| *Fumaria officinalis* L. | 2.2 | 0.7 | 3.6 | 90.4 |
| *Lolium multiflorum* Lam. | 4.6 | 0.5 | 2.8 | 93.1 |
| *Amaranthus retroflexus* L. | 9.4 | 0.3 | 1.8 | 95.0 |
| *Lolium perenne* L. | 1.4 | 0.2 | 1.1 | 96.1 |
| *Euphorbia helioscopia* L. subsp. *heliscopia* | 2.2 | 0.1 | 0.7 | 96.8 |
| *Stachys annua* L. subsp. *annua* | 1.9 | 0.1 | 0.5 | 97.4 |
| Conventional – RC + LG (n=12) | | | | |
| *Convolvulus arvensis* L. | 8.7 | 7.9 | 37.3 | 37.3 |
| *Cirsium arvense* (L.) Scop. | 3.2 | 4.1 | 19.4 | 56.6 |
| *Sorghum halepense* (L.) Pers. | 6.9 | 2.5 | 11.8 | 68.5 |
| *Xanthium orientale* L. | 7.2 | 1.8 | 8.6 | 77.1 |
| *Lolium perenne* L. | 1.7 | 1.5 | 7.2 | 84.3 |
| *Xanthium spinosum* L. | 4.0 | 0.9 | 4.2 | 88.4 |
| *Euphorbia prostrata* Aiton | 0.9 | 0.5 | 2.1 | 90.5 |

| Species | Average abundance | Average similarity | Contribution to group similarity (%) | Cumulative contribution (%) |
|---|---|---|---|---|
| *Setaria italica* (L.) P.Beauv. subsp. *viridis* (L.) Thell. | 1.1 | 0.3 | 1.3 | 91.8 |
| *Amaranthus retroflexus* L. | 0.3 | 0.2 | 1.1 | 92.9 |
| *Veronica persica* Poir. | 1.7 | 0.2 | 1.0 | 93.9 |
| *Digitaria sanguinalis* (L.) Scop. | 0.9 | 0.2 | 0.8 | 94.7 |
| *Mentha suaveolens* Ehrh. | 1.2 | 0.2 | 0.7 | 95.4 |

### 9.1.9 Figures

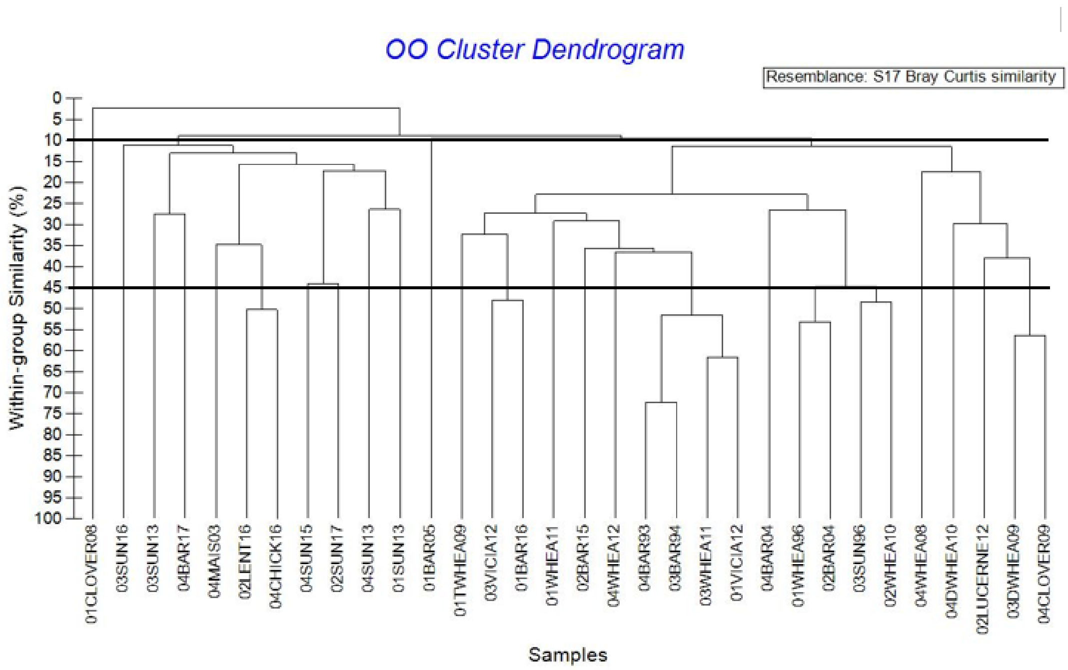


Figure S27: Cluster dendrogram grouping sample crop-weed communities of the old organic (OO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Valdipesa, Florence, Tuscany, in the period 1993-2017. Results were obtained after standardization by percentage of the species variables and calculation of a similarity matrix based on the Bray–Curtis coefficient. Sample labels include information on field, crop and time of observation, respectively. Two major groupings were identified at 10% of within-group similarity. Cluster composition at 45% of within-group similarity was used to complement multi-dimensional scaling ordination considering four categories of crops, i.e. winter cereals, row crops, legume crops for forage and for grain.

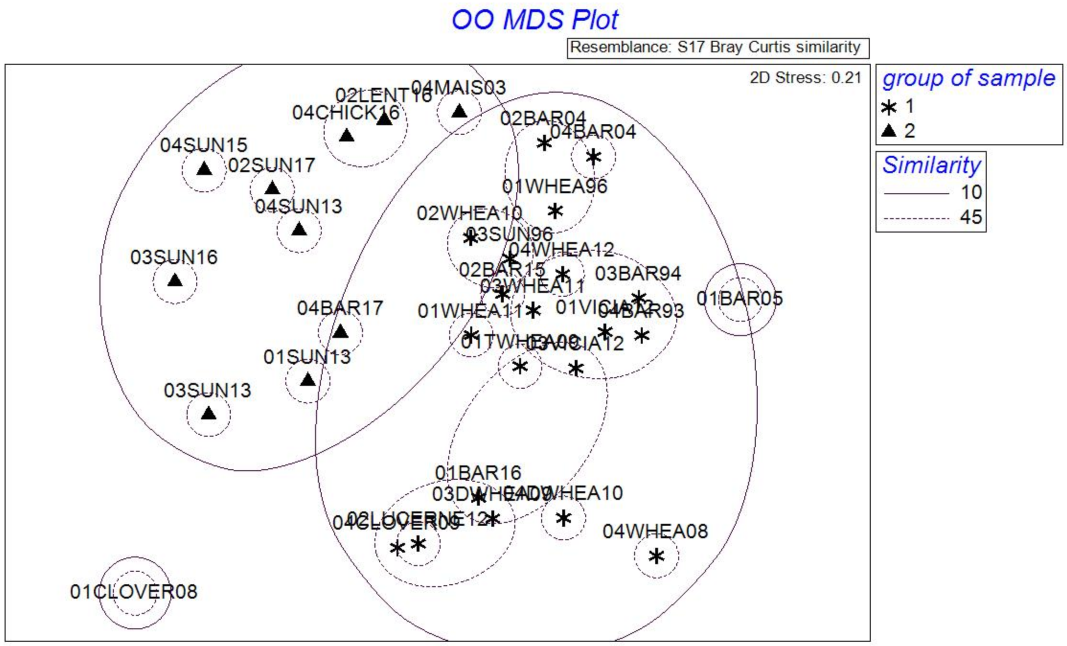


Figure S28: Superimposition of cluster groupings on the multi-dimensional scaling plot representing crop-weed communities of the old organic (OO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Valdipesa, Florence, Tuscany, in the period 1993-2017. Results were obtained after standardization by percentage of the variables and calculation of a similarity matrix based on the Bray–Curtis coefficient. Sample labels include information on field, crop and time of observation, respectively. The stress value of the representation is 0.21. Two major groups were identified, i.e. Group 1 (labelled with a star), including mainly winter cereals and legume crops for forage, and Group 2 (labelled with a triangle), including mainly row crops and legume crops for grain.

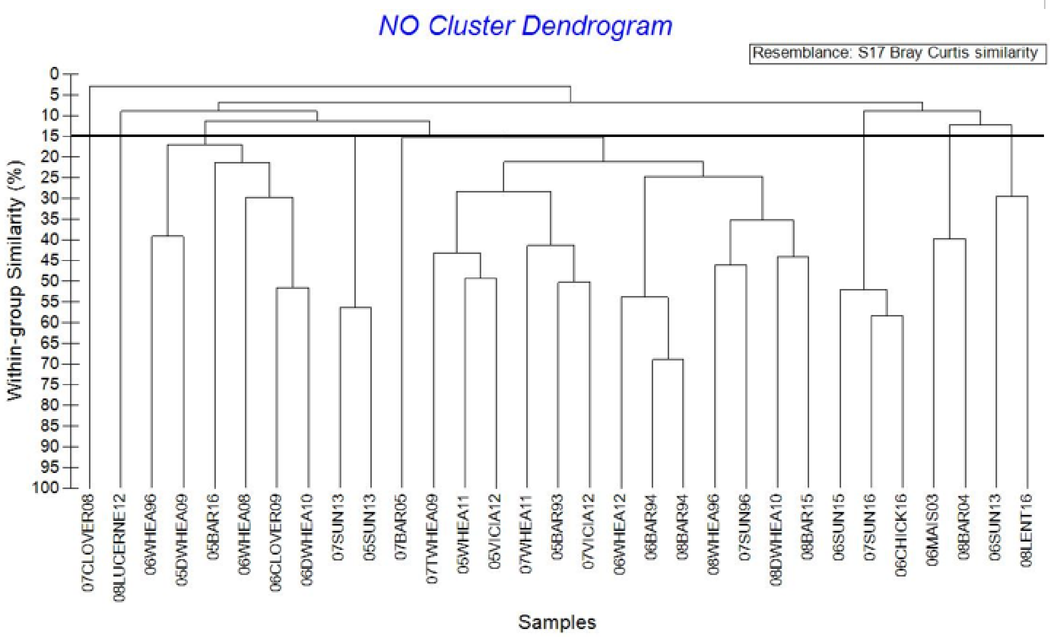


Figure S29: Cluster dendrogram grouping sample crop-weed communities of the new organic (NO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. Results were obtained after standardization by percentage of the species variables and calculation of a similarity matrix based on the Bray–Curtis coefficient. Sample labels include information on field, crop and time of observation, respectively. Six groupings and two out-layers were identified at 15% of within-group similarity.

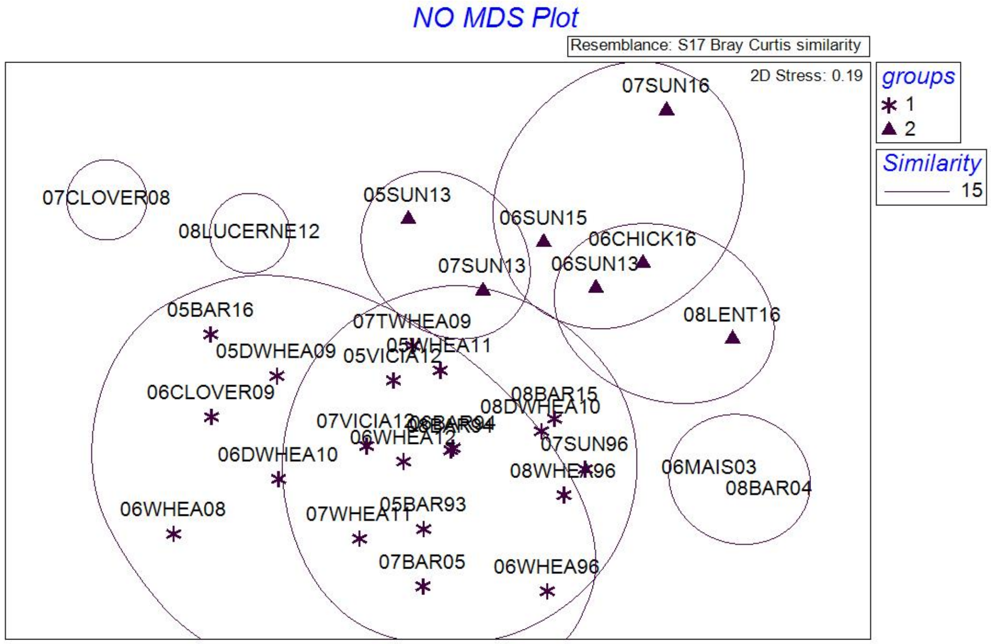


Figure S30: Superimposition of cluster groupings on the multi-dimensional scaling plot representing crop-weed communities of the new organic (NO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. Results were obtained after standardization by percentage of the variables and calculation of a similarity matrix based on the Bray–Curtis coefficient. Sample labels include information on field, crop and time of observation, respectively. The stress value of the representation is 0.19. Two major groups were identified, i.e. Group 1 (labelled with a star), including mainly winter cereals and legume crops for forage, and Group 2 (labelled with a triangle), including row crops and legume crops for grain.

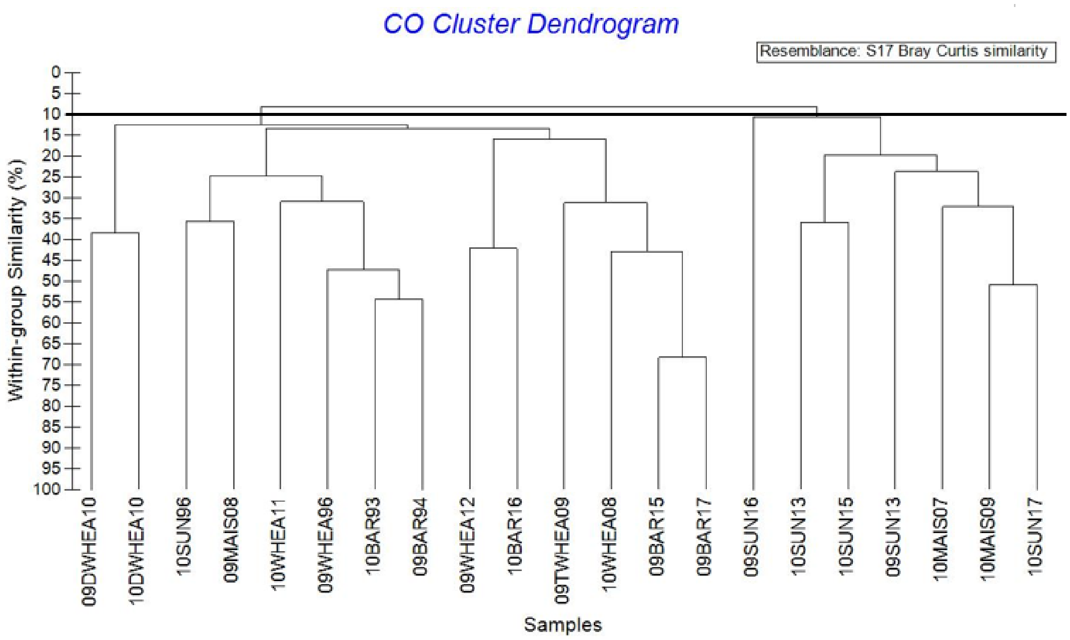


Figure S31: Cluster dendrogram grouping sample crop-weed communities of the conventional (CO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. Results were obtained after standardization by percentage of the species variables and calculation of a similarity matrix based on the Bray–Curtis coefficient. Sample labels include information on field, crop and time of observation, respectively. Two groups were identified at 10% of within-group similarity.

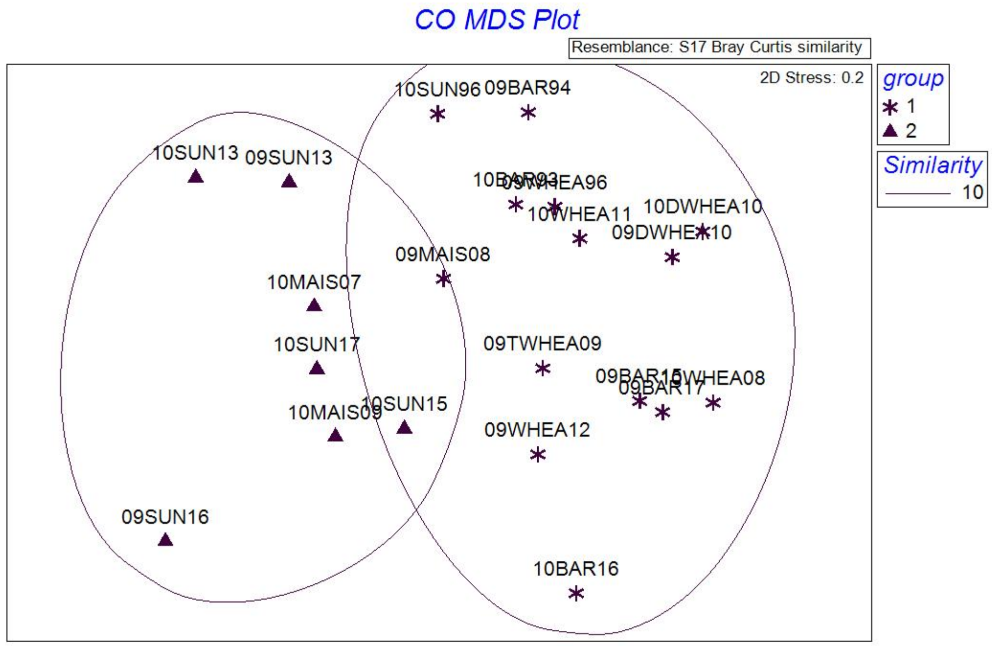


Figure S32: Superimposition of cluster groupings on the multi-dimensional scaling plot representing crop-weed communities of the conventional (CO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. Results were obtained after standardization by percentage of the variables and calculation of a similarity matrix based on the Bray–Curtis coefficient. Sample labels include information on field, crop and time of observation, respectively. The stress value of the representation is 0.20. Two major groups were identified, i.e. Group 1 (labelled with a star), including mainly winter cereals, and Group 2 (labelled with a triangle), including row crops.

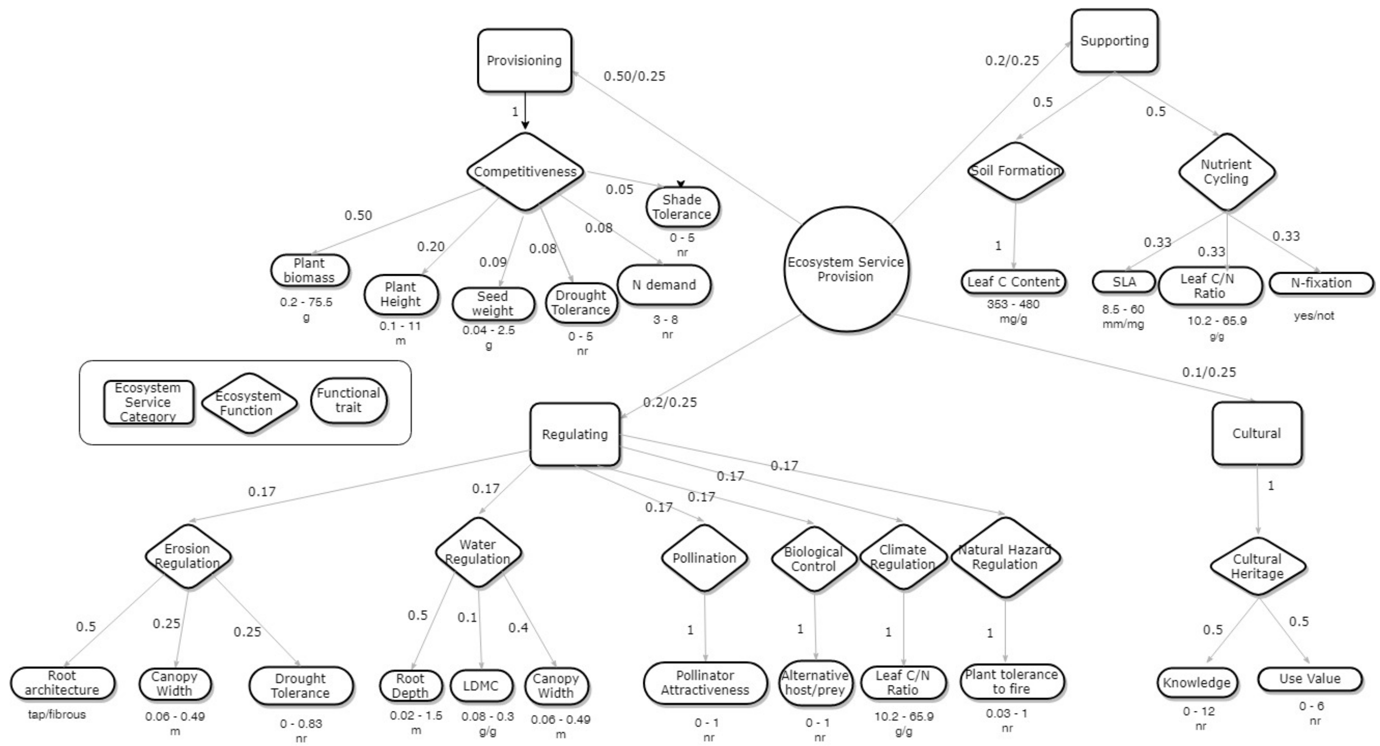


Figure S33: FunBies model structure. FunBies was applied to each species of the most characterizing weeds of organic and conventional micro-agroecosystems at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany. For each ecosystem service (ES) category (rectangles), corresponding ES category alternative weights, ecosystem functions (EFs, diamonds), EF weights, functional trait (FTs, ellipses), FT weights and FT score ranges are reported. Legend: SLA, specific leaf area; LDMC, leaf dry matter content.

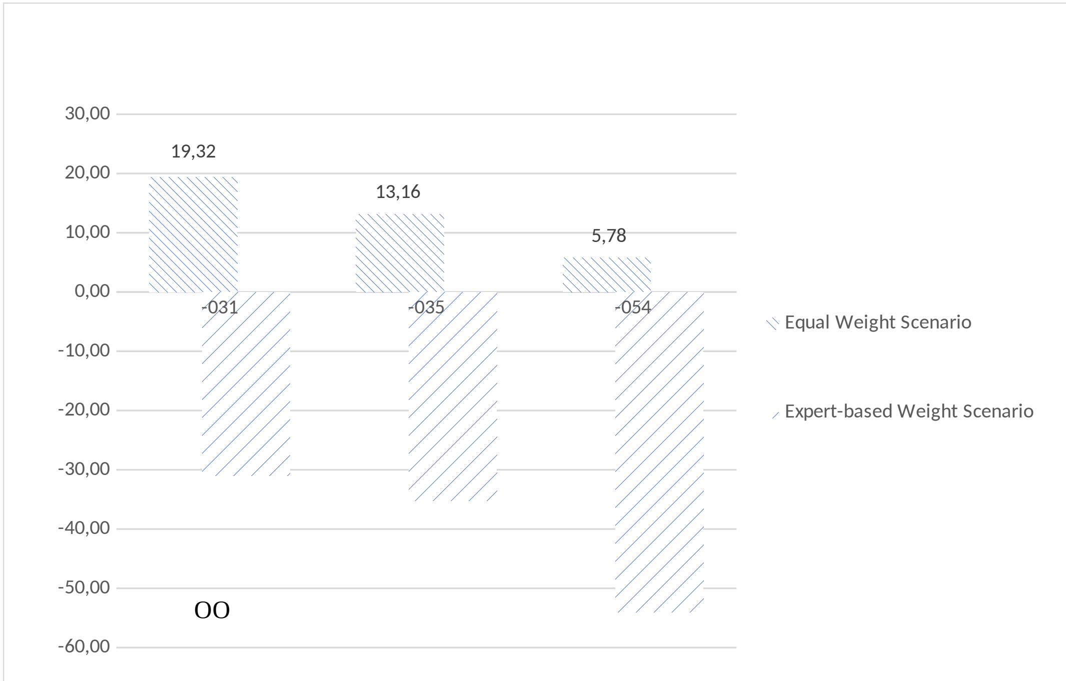


Figure S34: Yearly averages of overall ecosystem service (ES) provision provided by representative crop-weed communities of the old organic (OO), new organic (NO) and conventional (CO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. ES provision was calculated under two scenarios: equal weight scenario and expert-based weight scenario. Experts proposed weights as follows: 0.5 for the provisioning category, 0.2 for the regulating and supporting categories and 0.1 for the cultural category.

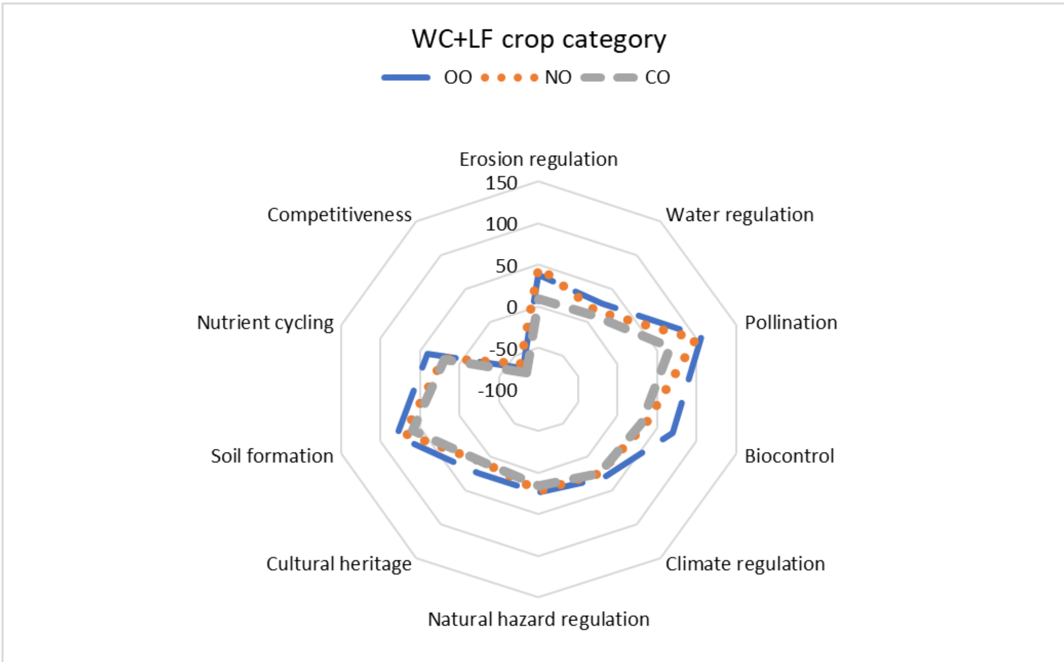


Figure S35: Provision of ecosystem services by representative crop-weed communities of winter crops + legume crops for forage (WC+LF) category groups in the old organic (OO), new organic (NO) and conventional (CO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. In FunBies we consider that regulating services are supplied by a number of ecosystem functions including erosion regulation, water regulation, pollination, biocontrol, climate regulation and natural hazard regulation; cultural services are supplied by cultural heritage; supporting services are supplied by soil formation and nutrient cycling; competitiveness represents the ability of weeds to generate a negative impact on provisioning services.

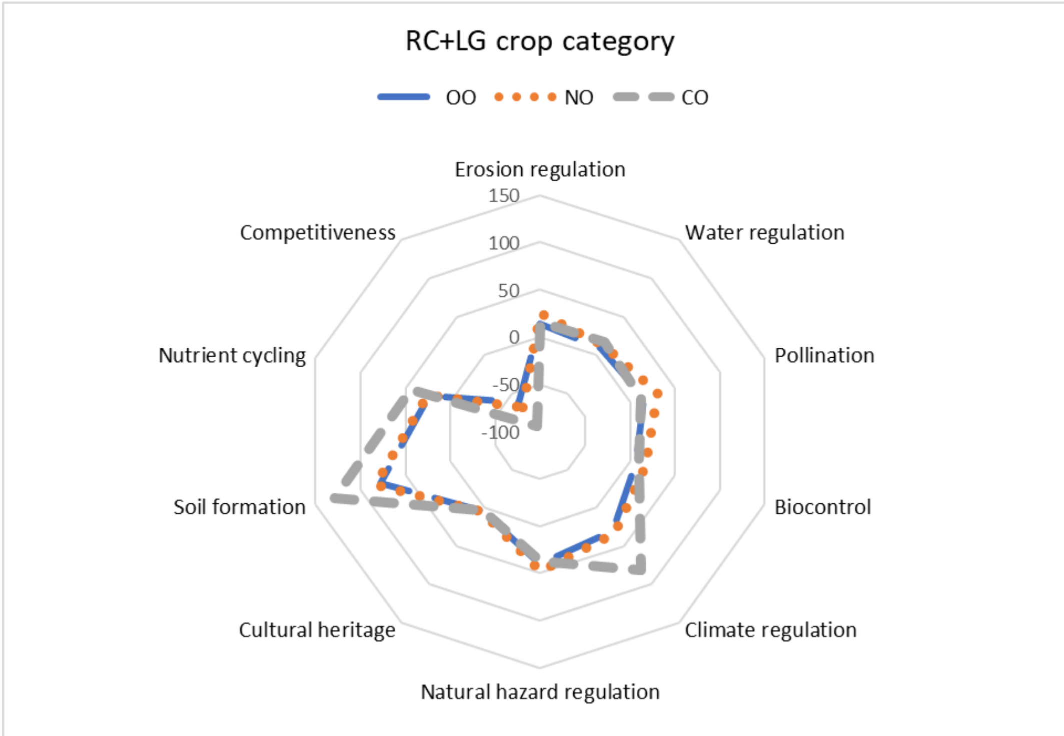


Figure S36: Provision of ecosystem services by representative crop-weed communities of row crops + legume crops for grain (RC+LG) category groups in the old organic (OO), new organic (NO) and conventional (CO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. In FunBies we consider that regulating services are supplied by a number of ecosystem functions including erosion regulation, water regulation, pollination, biocontrol, climate regulation and natural hazard regulation; cultural services are supplied by cultural heritage; supporting services are supplied by soil formation and nutrient cycling; competitiveness represents the ability of weeds to generate a negative impact on provisioning services.

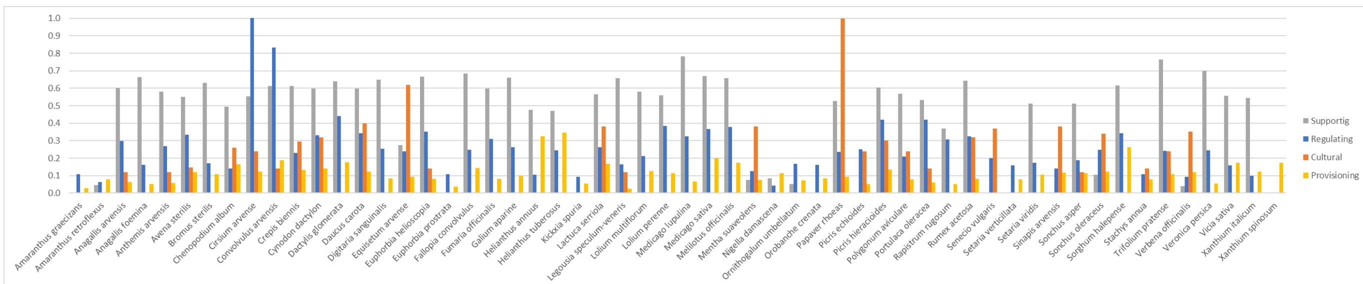


Figure S37: Supporting, regulating, cultural and provisioning services supplied by each single species collected in the old organic, new organic and conventional micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. The species scores of the ecosystem service categories range between 0 and 1 and represent the contribution to functional biodiversity of either a single individual or of a single unit of dry matter weight. Category scores result from sequential, linear additive aggregation of standardized scores of functional trait indicators. The more abundant is a species in a field, in a hectare or in whatever reference area, the more it can contribute to ecosystem services and overall functional biodiversity. Provisioning (dis)service due to competitiveness is reported in absolute terms; indeed species values hold negative impacts on functional biodiversity.

### 9.1.10 References


Ackerly DD (2003) Community Assembly, Niche Conservatism, and Adaptive Evolution in Changing Environments. International Journal of Plant Sciences 164:S165–S184. https://doi.org/10.1086/368401

Altieri MA (1999) The ecological role of biodiversity in agroecosystems. Agriculture, Ecosystems & Environment 74:19–31. https://doi.org/10.1016/S0167-8809(99)00028-6

Aminpanah H (2013) Competitive ability of canola cultivars (Brassica napus L.) against their natural weed populations. International Journal of Biosciences (IJB) 3:121–128. https://doi.org/10.12692/ijb/3.3.121-128

Bagstad KJ, Semmens DJ, Waage S, Winthrop R (2013) A comparative assessment of decision-support tools for ecosystem services quantification and valuation. Ecosystem Services 5:27–39. https://doi.org/10.1016/j.ecoser.2013.07.004

Balzan MV, Moonen A-C (2014) Field margin vegetation enhances biological control and crop damage suppression from multiple pests in organic tomato fields. Entomologia Experimentalis et Applicata 150:45–65. https://doi.org/10.1111/eea.12142

Boerema A, Rebelo AJ, Bodi MB, Esler KJ, Meire P (2017) Are ecosystem services adequately quantified? Journal of Applied Ecology 54:358–370. https://doi.org/10.1111/1365-2664.12696

Bonanomi G, Motti R, De Marco A, Idbella M (2023) Temperature sensitivity and decomposition rate of 101 leaf litter types from Mediterranean ecosystems. Science of The Total Environment 894:165026. https://doi.org/10.1016/j.scitotenv.2023.165026

Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecological monographs 27:326–349

Camangi F, Stefani A, Uncini Manganelli R, Tomei P, Trimarchi S, Oggiano N, Loni A (2007) L'uso delle erbe nella tradizione rurale della Toscana

Chapin III FS, Bret-Harte MS, Hobbie SE, Zhong H (1996) Plant functional types as predictors of transient responses of arctic vegetation to global change. Journal of Vegetation Science 7:347–358. https://doi.org/10.2307/3236278

Chatzinikolaou P, Raggi M, Viaggi D (2015) The evaluation of Ecosystem Services production: An application in the Province of Ferrara. Bio-based and Applied Economics Journal 4:235–259. https://doi.org/10.22004/ag.econ.231940

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Austral Ecology 18:117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Clarke KR, Warwick R (2001) Change in marine communities. An approach to statistical analysis and interpretation 2:1–68

Corsi G, Pagni AM (1979) Piante selvatiche di uso alimentare in Toscana. In: Piante selvatiche di uso alimentare in Toscana. Pacini, p 199

Costanza R, de Groot R, Braat L, Kubiszewski I, Fioramonti L, Sutton P, Farber S, Grasso M (2017) Twenty years of ecosystem services: How far have we come and how far do we still need to go? Ecosystem Services 28:1–16. https://doi.org/10.1016/j.ecoser.2017.09.008

Costanza R, Folke C (1997) Valuing ecosystem services with efficiency, fairness and sustainability as goals. Nature's services: Societal dependence on natural ecosystems 49–70

Costanzo A, Bàrberi P (2014) Functional agrobiodiversity and agroecosystem services in sustainable wheat production. A review. Agronomy for Sustainable Development 34:327–348. https://doi.org/10.1007/s13593-013-0178-1

Craine JM, Dybzinski R (2013) Mechanisms of plant competition for nutrients, water and light. Functional Ecology 27:833–840. https://doi.org/10.1111/1365-2435.12081

Craine JM, Froehle J, Tilman DG, Wedin DA, Chapin FS Iii (2001) The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. Oikos 93:274–285. https://doi.org/10.1034/j.1600-0706.2001.930210.x

Cunningham SA, Summerhayes B, Westoby M (1999) Evolutionary Divergences in Leaf Structure and Chemistry, Comparing Rainfall and Soil Nutrient Gradients. Ecological Monographs 69:569–588. https://doi.org/10.1890/0012-9615(1999)069%5B0569:EDILSA%5D2.0.CO;2

De Baets S, Poesen J, Knapen A, Barberá GG, Navarro JA (2007) Root characteristics of representative Mediterranean plant species and their erosion-reducing potential during concentrated runoff. Plant and Soil 294:169–183. https://doi.org/10.1007/s11104-007-9244-2

De Baets S, Poesen J, Meersmans J, Serlet L (2011) Cover crops and their erosion-reducing effects during concentrated flow erosion. CATENA 85:237–244. https://doi.org/10.1016/j.catena.2011.01.009

de Groot RS (1992) Functions of nature : Evaluation of nature in environmental planning, management and decision making. Functions of nature : evaluation of nature in environmental planning, management and decision making 315

de Groot RS, Alkemade R, Braat L, Hein L, Willemen L (2010) Challenges in integrating the concept of ecosystem services and values in landscape planning, management and decision making. Ecological Complexity 7:260–272. https://doi.org/10.1016/j.ecocom.2009.10.006

De Groot RS, Wilson MA, Boumans RM (2002) A typology for the classification, description and valuation of ecosystem functions, goods and services. Ecological economics 41:393–408

Diaz S, Cabido M (1997) Plant functional types and ecosystem function in relation to global change. Journal of Vegetation Science 8:463–474. https://doi.org/10.2307/3237198

Diaz S, Hodgson Jg, Thompson K, et al (2004) The plant traits that drive ecosystems: Evidence from three continents. Journal of Vegetation Science 15:295–304. https://doi.org/10.1111/j.1654-1103.2004.tb02266.x

Dodgson JS, Spackman M, Pearman A, Phillips LD (2009) Multi-criteria analysis: A manual. Communities and Local Government, Wetherby

Durka W (2002) Blüten- und Reproduktionsbiologie. In: BIOLFLOR: Eine Datenbank mit biologisch-ökologischen Merkmalen zur Flora von Deutschland. Bundesamt für Naturschutz Bonn, pp 133–175

Ecoflora (2022) Ecological flora of Britain and Ireland. ecoflora.org.uk

Egoh B, Drakou EG, Dunbar MB, Maes J, Willemen L (2012) Indicators for mapping ecosystem services: A review. Publications Office of the European Union, LU

Egoh B, Rouget M, Reyers B, Knight AT, Cowling RM, van Jaarsveld AS, Welz A (2007) Integrating ecosystem services into conservation assessments: A review. Ecological Economics 63:714–721. https://doi.org/10.1016/j.ecolecon.2007.04.007

Esposito M, Westbrook AS, Maggio A, Cirillo V, DiTommaso A (2023) Neutral weed communities: The intersection between crop productivity, biodiversity, and weed ecosystem services. Weed Science 71:301–311. https://doi.org/10.1017/wsc.2023.27

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD (2004) Pollination Syndromes and Floral Specialization. Annual Review of Ecology, Evolution, and Systematics 35:375–403. https://doi.org/10.1146/annurev.ecolsys.34.011802.132347

Frassinelli N (2008) Indagini sulle conoscenze etnobotaniche a Sant'Agata (Scarperia, FI). Master's thesis, Università degli Studi di Firenze

Freschet GT, Cornelissen JHC, van Logtestijn RSP, Aerts R (2010) Evidence of the "plant economics spectrum" in a subarctic flora. Journal of Ecology 98:362–373. https://doi.org/10.1111/j.1365-2745.2009.01615.x

Gabriel D, Tscharntke T (2007) Insect pollinated plants benefit from organic farming. Agriculture, Ecosystems & Environment 118:43–48. https://doi.org/10.1016/j.agee.2006.04.005

Gatto P, Vidale E, Secco L, Pettenella D (2013) Exploring the willingness to pay for forest ecosystem services by residents of the Veneto Region. Bio-based and Applied Economics 21–43 Pages. https://doi.org/10.13128/BAE-11151

Gibson RH, Nelson IL, Hopkins GW, Hamlett BJ, Memmott J (2006) Pollinator webs, plant communities and the conservation of rare plants: Arable weeds as a case study: Pollinator webs and rare plant conservation. Journal of Applied Ecology 43:246–257. https://doi.org/10.1111/j.1365-2664.2006.01130.x

Gorley K, Clarke K (2006) PRIMER v6: User manual/tutorial. PRIMER-E, Plymouth, UK

Grime JP (1998) Benefits of plant diversity to ecosystems: Immediate, filter and founder effects. Journal of Ecology 86:902–910. https://doi.org/10.1046/j.1365-2745.1998.00306.x

Grime JP, Thompson K, Hunt R, et al (1997) Integrated Screening Validates Primary Axes of Specialisation in Plants. Oikos 79:259. https://doi.org/10.2307/3546011

Gyssels G, Poesen J (2003) The importance of plant root characteristics in controlling concentrated flow erosion rates. Earth Surface Processes and Landforms 28:371–384. https://doi.org/10.1002/esp.447

Hill MO, Mountford JO, Roy DB, Bunce RGH (1999) Ellenberg's indicator values for British plants. ECOFACT Volume 2 Technical Annex. Institute of Terrestrial Ecology

Hodgson JG, Montserrat-Martí G, Charles M, et al (2011) Is leaf dry matter content a better predictor of soil fertility than specific leaf area? Annals of Botany 108:1337–1345. https://doi.org/10.1093/aob/mcr225

Istituto Nazionale di Statistica (ISTAT) (2010) 6° Censimento dell'agricoltura. ISTAT

Istituto Regionale Programmazione Economica della Toscana (IRPET) (2015) PSR 2014-2020. Repertorio giustificativi premi. IRPET

Ivey CT, Carr DE (2005) Effects of herbivory and inbreeding on the pollinators and mating system of Mimulus guttatus (Phrymaceae). American Journal of Botany 92:1641–1649. https://doi.org/10.3

732/ajb.92.10.1641

Köbrich C, Rehman T, Khan M (2003) Typification of farming systems for constructing representative farm models: Two illustrations of the application of multi-variate analyses in Chile and Pakistan. Agricultural systems 76:141–157

Lavorel S (2013) Plant functional effects on ecosystem services. Journal of Ecology 101:4–8. https://doi.org/10.1111/1365-2745.12031

Lavorel S, Garnier E (2002) Predicting changes in community composition and ecosystem functioning from plant traits: Revisiting the Holy Grail: *Plant Response and Effect Groups*. Functional Ecology 16:545–556. https://doi.org/10.1046/j.1365-2435.2002.00664.x

Lavorel S, Harrington R, Storkey J, Dıaz S, De Bello F, Bardgett R, Dolédec S, Feld C, Roux X, Berg M (2009) RUBICODE—how trait linkages within and across trophic levels underlie the vulnerability of ecosystem services. FP6, Thematic Area: Global Change and Ecosystems European Commission, DG Research, Bruxelles

Lochon I, Colace M-P, Devaux C, Grigulis K, Rettinger R, Lavorel S (2018) Taxonomic and functional facets of the resilience to management of mown subalpine grasslands. Applied Vegetation Science 21:636–646. https://doi.org/10.1111/avsc.12392

Lundgren JG (2009) Relationships of natural enemies and non-prey foods. Springer, Dordrecht

Millennium Ecosystem Assessment (2005) Ecosystems and human well-being: synthesis. Island Press, Washington, DC

Mitchell RJ, Karron JD, Holmquist KG, Bell JM (2004) The influence of Mimulus ringens floral display size on pollinator visitation patterns. Functional Ecology 18:116–124. https://doi.org/10.1111/j.1365-2435.2004.00812.x

Molina-Montenegro MA, Cavieres LA (2006) Effect of density and flower size on the reproductive success of Nothoscordum graminum (Alliaceae). Gayana Botánica 63: https://doi.org/10.4067/S0717-66432006000100004

Molines MTE (2018) Etnobotánica en el Alto Valle del Reno (Toscana y Emilia-Romaña, Italia): Etnobotanica nell'Alta Valle del Reno (Toscana ed Emilia-Romagna, Italia). Firenze University Press

Moonen A-C, Bàrberi P (2008) Functional biodiversity: An agroecosystem approach. Agriculture, Ecosystems & Environment 127:7–21. https://doi.org/10.1016/j.agee.2008.02.013

Müller H (1883) The fertilisation of flowers. Macmillan

Nardo M, Saisana M (2005) Handbook on Constructing Composite Indicators: Methodology and User Guide. Joint Research Centre-European Commission

Noble BF (2004) Strategic environmental assessment quality assurance: Evaluating and improving the consistency of judgments in assessment panels. Environmental Impact Assessment Review 24:3–25

Oikonomou V, Dimitrakopoulos PG, Troumbis AY (2011) Incorporating Ecosystem Function Concept in Environmental Planning and Decision Making by Means of Multi-Criteria Evaluation: The Case-Study of Kalloni, Lesbos, Greece. Environmental Management 47:77–92. https://doi.org/10.1007/s00267-010-9575-2

Pacini GC, Merante P, Lazzerini G, Van Passel S (2015) Increasing the cost-effectiveness of EU agrienvironment policy measures through evaluation of farm and field-level environmental and economic performance. Agricultural Systems 136:70–78. https://doi.org/10.1016/j.agsy.2015.02.004

Pakeman RJ, Eastwood A, Scobie A (2011) Leaf dry matter content as a predictor of grassland litter decomposition: A test of the "mass ratio hypothesis." Plant and Soil 342:49–57. https://doi.org/10.1007/s11104-010-0664-z

Paracchini ML, Pacini C, Jones MLM, Pérez-Soba M (2011) An aggregation framework to link indicators associated with multifunctional land use to the stakeholder evaluation of policy options. Ecological Indicators 11:71–80. https://doi.org/10.1016/j.ecolind.2009.04.006

Paula S, Arianoutsou M, Kazanis D, et al (2009) Fire-related traits for plant species of the Mediterranean Basin: *Ecological Archives* E090-094. Ecology 90:1420–1420. https://doi.org/10.1890/08-1309.1

Power AG (2010) Ecosystem services and agriculture: Tradeoffs and synergies. Philosophical Transactions of the Royal Society B: Biological Sciences 365:2959–2971. https://doi.org/10.1098/rstb.2010.0143

Price PW (2011) Insect Ecology: Behavior, Populations and Communities. Cambridge University Press, Cambridge

Qureshi ME, Harrison SR, Wegener MK (1999) Validation of multicriteria analysis models. Agricultural Systems 62:105–116. https://doi.org/10.1016/S0308-521X(99)00059-1

Randellini L (2007) L'uso delle piante nella tradizione popolare dell'alto Casentino. Master's thesis, Università degli Studi di Firenze

Reich PB (2014) The world-wide "fast-slow" plant economics spectrum: A traits manifesto. Journal of Ecology 102:275–301. https://doi.org/10.1111/1365-2745.12211

Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: Global convergence in plant functioning. Proceedings of the National Academy of Sciences 94:13730–13734. https://doi.org/10.1073/pnas.94.25.13730

Reubens B, Poesen J, Danjon F, Geudens G, Muys B (2007) The role of fine and coarse roots in shallow slope stability and soil erosion control with a focus on root system architecture: A review. Trees 21:385–402. https://doi.org/10.1007/s00468-007-0132-4

Ring I, Hansjürgens B, Elmqvist T, Wittmer H, Sukhdev P (2010) Challenges in framing the economics of ecosystems and biodiversity: The TEEB initiative. Current Opinion in Environmental Sustainability 2:15–26. https://doi.org/10.1016/j.cosust.2010.03.005

Schervish MJ (1987) A review of multivariate analysis. Statistical Science 2:396–413

Schirpke U, Kohler M, Leitinger G, Fontana V, Tasser E, Tappeiner U (2017) Future impacts of changing land-use and climate on ecosystem services of mountain grassland and their resilience. Ecosystem Services 26:79–94. https://doi.org/10.1016/j.ecoser.2017.06.008

Signorini MA, Lombardini C, Bruschi P, Vivona L (2007) Conoscenze etnobotaniche e saperi tradizionali nel territorio di San Miniato (Pisa). In: Atti Società Toscana di Scienze Naturali Memorie Serie B. pp 65–83

Smart SM, Glanville HC, Blanes MDC, Mercado LM, Emmett BA, Jones DL, Cosby BJ, Marrs RH, Butler A, Marshall MR, Reinsch S, Herrero-Jáuregui C, Hodgson JG (2017) Leaf dry matter content is better at predicting above-ground net primary production than specific leaf area. Functional Ecology 31:1336–1344. https://doi.org/10.1111/1365-2435.12832

Storkey J (2006) A functional group approach to the management of UK arable weeds to support biological diversity. Weed Research 46:513–522. https://doi.org/10.1111/j.1365-3180.2006.00528.x

Taylor BR, Parkinson D, Parsons WFJ (1989) Nitrogen and Lignin Content as Predictors of Litter Decay Rates: A Microcosm Test. Ecology 70:97–104. https://doi.org/10.2307/1938416

Torner C, Sanchez del Arco MJ, Satorre E, Fernandez-Quintanilla C (2000) A comparison of the growth patterns and the competitive ability of four annual weeds. Agronomie 20:147–156. https://doi.org/10.1051/agro:2000115

Wardle DA (1998) Controls of temporal variability of the soil microbial biomass: A global-scale synthesis. Soil Biology and Biochemistry 30:1627–1637. https://doi.org/10.1016/S0038-0717(97)00201-0

Woodcock BA, Garratt MPD, Powney GD, et al (2019) Meta-analysis reveals that pollinator functional diversity and abundance enhance crop pollination and yield. Nature Communications 10:1481. https://doi.org/10.1038/s41467-019-09393-6

Wright IJ, Reich PB, Westoby M, et al (2004) The worldwide leaf economics spectrum. Nature 428:821–827. https://doi.org/10.1038/nature02403

Wright IJ, Westoby M, Reich PB (2002) Convergence towards higher leaf mass per area in dry and nutrient-poor habitats has different consequences for leaf life span. Journal of Ecology 90:534–543. https://doi.org/10.1046/j.1365-2745.2002.00689.x

You X, Liu J, Zhang L (2015) Ecological modeling of riparian vegetation under disturbances: A review. Ecological Modelling 318:293–300. https://doi.org/10.1016/j.ecolmodel.2015.07.002

Zakharova L, Meyer KM, Seifan M (2019) Trait-based modelling in ecology: A review of two decades of research. Ecological Modelling 407:108703. https://doi.org/10.1016/j.ecolmodel.2019.05.008

Zhang G, Tang K, Ren Z, Zhang X-C (2013) Impact of Grass Root Mass Density on Soil Detachment Capacity by Concentrated Flow on Steep Slopes. Transactions of the ASABE 927–934. https://doi.org/10.13031/trans.56.9566

Zhang W, Ricketts TH, Kremen C, Carney K, Swinton SM (2007) Ecosystem services and dis-services to agriculture. Ecological Economics 64:253–260. https://doi.org/10.1016/j.ecolecon.2007.02.024

**Appendix A**

Table S31: Time schedule for species sampling.

| Month | Crops | Semi-natural habitats[1] |
|---|---|---|
| April (before first cutting) | *Medicago sativa* L. | First check |
| | *Trifolium squarrosum* L. | |
| | *Trifolium pratense* L. | |
| | *Trifolium alexandrinum* L. | |
| April-May | *Triticum durum* L. | |
| | *Triticum aestivum* L. | |
| | *Hordeum vulgare* L. | |
| May-June | *Zea mays* L. | Second check |
| | *Heliantus annuus* L. | |
| | *Vicia faba minor* L. | |
| | *Vicia lens* L. | |
| | *Cicer arietinum* L. | |

[1] Verges, ditch edges, areas around hedges and trees, permanent pastures, long duration leys after first cutting, set-aside.

Table S30: Ordinary weeding, fertilization and tillage operations at the MoLTE experiment. Legume crops were used only in the organic micro-agroecosystems.

| Crop type | Winter Cereals (WC) | | | Row Crops (RC) | | | Legumes for Grain (LG) Legumes for Forage (LF) | |
|---|---|---|---|---|---|---|---|---|
| Crop management option | OO | NO | CO | OO | NO | CO | OO | NO |
| Primary tillage[1] | Plowing or chisel plowing | Plowing or chisel plowing | Plowing or chisel plowing | Plowing or chisel plowing | Plowing or chisel plowing | Plowing or chisel plowing | Plowing or chisel plowing | Plowing or chisel plowing |
| Pre-sowing fertilization[1] | Green manure or organic fertilizer | Green manure or organic fertilizer | Diammonium phosphate | Green manure or organic fertilizer | Green manure or organic fertilizer | - | - | - |
| First fertilization[1] | - | - | Ammonium nitrate | - | - | 20.10.10 | - | - |
| Second fertilization[1] | - | - | Urea | - | - | Urea | - | - |
| Chemical weeding[1] | - | - | Axial (a.i. pinoxaden 10.6% and cloquintocetmexyl 2.55%) Axial Pronto (a.i. pinoxaden 6.4% and cloquintocetmexyl 1.55%) + Logran (a.i. triasulfuron 20%) | - | - | GOAL (a.i. oxyfluorfen) | - | - |
| Mechanical weeding[1] | Weed harrowing | Weed harrowing | Weed harrowing | Weed hoeing | Weed hoeing | Weed hoeing | - | - |

[1] During the 25-years of MoLTE experiment, ordinary agronomic operations could change due to year-specific production and climatic condition.

## Appendix B

Ecosystem functions (EFs), functional traits, (dis)services and corresponding descriptions included in the FunBies model, reported for each of the Millennium Ecosystem Assessment (MA) EF categories.

Table S32: Ecosystem function, functional traits, disservice and corresponding descriptions of the MA provisioning category

| Ecosystem function | Functional trait | Disservice | Description |
|---|---|---|---|
| Production of biomass | Dry Matter Biomass | Competitiveness with the main crop | A larger weed biomass results in higher competitiveness with the main crop |
| | Canopy height | | The higher the weed height, the lighter weeds capture (and less the main crop) |
| | Shading tolerance | | A higher shading tolerance means more tolerance towards the shading crop and hence more competitiveness |
| | Nitrogen requirement | | Higher N required means higher competitiveness especially in N-poor conditions |
| | Drought tolerance | | Higher drought tolerance means higher competitiveness especially in dry conditions |
| | Seed weight | | Competitive effect is associated with initial plant size (seed weight and rate of emergence) |

Table S33: Ecosystem function, functional traits, services and corresponding descriptions of the MA regulating category

| Ecosystem function | Functional trait | Service | Description |
|---|---|---|---|
| Erosion regulation | Root Architecture (tap/fibrous) | Prevention of damage from erosion | Fibrous roots have a better impact in soil erosion control |
| | Crown (canopy) width | | A wider canopy may cover a larger portion of soil while preventing soil erosion due to intensive rain |
| | Drought tolerance | | A plant which tolerates drought periods will be able to protect soil from erosion even in summer (when more intensive meteorological phenomenon occurs) |
| Water regulation | Root depth | Drainage, filtering and storage of water | Deeper roots perform better in maintaining a good soil structure and hence allowing water retention |
| | Crown (canopy) widt | | Plant canopy decreases the kinetic energy of drops that cause erosion |
| | Leaf dry matter content | | LDMC is the parameter that better predict organic matter decomposition |
| Pollination | Müller class for Flowering Phenology/ Richness for Flowering type | Pollination of wild plant species and crops | Role of pollinators in movement of floral gametes, weighted by the flowering period of each species and the type of flower (Eg. composite flowers are made up of inflorescences and each of them may provide pollen) |
| Biological control | Hosting pests | Control of pests and diseases | Plants more likely visited by pests likely support also natural enemies/predators |
| Climate regulation | Leaf C/N ratio | Carbon sequestration | It indicates the capability of organic matter to decompose ($CO_2$ fixed into the soil) instead of emitting $CO_2$ into the atmosphere) |
| Natural hazard regulation | Plant tolerance to fire | Role of forests in dampening extreme events | Plants might reduce fire damages with their structural characteristics |

Table S34: Ecosystem function, functional traits, services and corresponding descriptions of the MA supporting category

| Ecosystem function | Functional trait | Service | Description |
|---|---|---|---|
| Nutrient cycling | Nitrogen fixation | Nitrogen fixation into the soil | Leguminous species provide nitrogen to the system |
| | Leaf C/N ratio | Nitrification conditions | A good soil structure quality can lead to nitrification conditions and therefore favour nitrate formation |
| Soil formation | Leaf carbon content per leaf dry mass | Carbon supply from leaf decomposition | Carbon can be supplied to the soil from leaf decomposition |
| | Specific leaf area | Organic matter supply | It determines the rate and speed of organic matter decomposition |

Table S35: Ecosystem function, functional traits, services and corresponding descriptions of the MA cultural category

| Ecosystem function | Functional trait | Service | Description |
|---|---|---|---|
| Local memory of the use | Use reported in local literature | Preserve traditional knowledge | The citation of the use of a species in local literature is considered an indicator of the locals' memory |
| Cultural heritage | Presence of a species in local literature | Sense of place and identity | The citation of a species in local literature is considered an indicator of the traditional heritage value |

## Appendix C

Table S36: Müller classes with relative characteristics and the corresponding typical pollinators which differ in length of proboscis and corresponding scores.

| Müller Class[1] | Characteristic[1] | Typical Pollinators[1] | Score[2] |
|---|---|---|---|
| A | flowers with open nectar | beetles, flies, syrphids, wasps, medium tongued bees | 1.0 |
| AB | flowers with partly hidden nectar | syrphids, bees | 0.9 |
| B | flowers with totally hidden nectar | bees, bumblebees, wasps, bombylides, syrphids | 0.7 |
| B` | flower associations with totally hidden nectar | bees, bumble bees, wasps, bombylides, syrphids | 0.7 |
| H | hymenoptere flowers | hymenoptere | 0.5 |
| Hb | bee flowers | bees | 0.6 |
| Hh | bumble bee flowers | bumble bees | 0.4 |
| Hw | wasp flowers | wasps | 0.1 |
| Hi | ichneumonide flowers | ichneumonidae | 0.1 |
| F | butterfly flowers | butterflies, long tongued bees, syrphids | 0.3 |
| Ft | butterfly flowers | butterflies | 0.1 |
| Fn | moth flowers | moths | 0.05 |
| D | fly flowers | flies | 0.1 |
| De | nasty flowers | muscidae | 0.1 |
| Dke | trap flowers | very small dipteres | 0.05 |
| Dkl | clamp trap flowers | flies, bees | 0.05 |
| Dt | deceptive flowers | flies | 0.1 |
| Ds | syrphid flowers | syrphids | 0.2 |
| Kl | small insect flowers | small ichneumonide, flies, beetles | 0.3 |

| Müller Class[1] | Characteristic[1] | Typical Pollinators[1] | Score[2] |
|---|---|---|---|
| Po | pollen flowers | short tongued bees, syrphids, flies, beetles | 0.3 |
| W | wind flowers | - | 0.0 |
| Wb | wind flowers occassionally visited by insect | short tongued bees, syrphids, flies, beetles | 0.3 |
| Hy | water flowers: pollination on or under water | - | 0.0 |
| ABDe | transition type flowers with partly hidden nectar - nasty flowers | flies, beetles | 0.9 |
| AD | transition type flowers with open nectar - fly flowers | flies | 0.1 |
| ADe | transition type flowers with open nectar - nasty flowers | flies, beetles | 0.9 |
| B` | transition type flower associations with totally hidden nectar - butterfly flowers | bumble bees, lepidoptera | 0.3 |
| BD | transition type flowers with totally hidden nectar - fly flowers | flies | 0.1 |
| BF | transition type flowers with totally hidden nectar - butterfly flowers | bees, flies | 0.6 |
| BH | transition type flowers with totally hidden nectar - bee flowers | hymenopteres | 0.5 |
| BHb | transition type flowers with totally hidden nectar - bee flowers in a narrow sense | bees, tongue $< 7$ mm | 0.4 |
| BHh | transition type flowers with totally hidden nectar - bumble bee flowers | bees, tongue $> 7$ mm | 0.6 |

| Müller Class[1] | Characteristic[1] | Typical Pollinators[1] | Score[2] |
|---|---|---|---|
| BHw | transition type flowers with totally hidden nectar - wasp flowers | wasps | 0.1 |
| DsB | transition type syrphid flowers - flowers with totally hidden nectar | syrphids | 0.2 |
| FD | transition type butterfly flowers - fly flowers | lepidoptera, flies | 0.2 |
| FHb | transition type butterfly flowers - bee flowers in a narrow sense | lepidoptera, bees | 0.4 |
| FHh | transition type butterfly flowers - bumble bee flowers | lepidoptera, bumble bees | 0.3 |
| FnH | transition type moth flowers - bee flowers | moths, hymenoptera | 0.1 |
| HF | transition type bee flowers - butterfly flowers | bees, lepidoptera | 0.4 |
| HFt | transition type bee flowers - butterfly flowers | bees, butterflies | 0.4 |
| HhDs | transition type bumble bee flowers - syrphid flowers | bumblebees, syrphids | 0.3 |
| HhF | transition type bumble bee flowers - butterfly flowers | bumblebees, lepidoptera | 0.2 |
| HhFn | transition type bumble bee flowers - moth flowers | bumblebees, moths | 0.2 |
| HhFt | transition type bumble bee flowers - butterfly flowers | bumblebees, butterfflies | 0.2 |
| PoA | transition type pollen flowers - flowers with open nectar | beetles, flies, syrphids, wasps, medium tounged bees | 0.8 |
| PoAB | transition type pollen flowers - flowers with partly hidden nectar | beetles, flies, syrphids, wasps, medium tounged bees | 0.8 |

| Müller Class[1] | Characteristic[1] | Typical Pollinators[1] | Score[2] |
|---|---|---|---|
| PoDe | transition type pollen flowers - nasty flowers | short tongued bees, syrphids, muscids, beetles | 0.3 |
| PoWb | transition type pollen flowers - wind blossoms occassionally visited by insect | short tounged bees, syrphids, muscids, beetles | 0.3 |

[1]Durka(2002)
[2]Expert-based.

# Appendix D

Table S37: Ecosystem service (ES) provision by ES category for each of the species collected in the old organic (OO), new organic (NO) and conventional (CO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Valdipesa, Florence, Tuscany, in the period 1993-2017.

| Species | Provisioning | Regulating | Supporting | Cultural | FBI |
|---|---|---|---|---|---|
| *Amaranthus graecizans* L. | -0.03 | 0.11 | 0.00 | 0.00 | -0.02 |
| *Amaranthus retroflexus* L. | -0.08 | 0.06 | 0.05 | 0.00 | 0.02 |
| *Lysimachia arvensis* (L.) U. Manns & Anderb. | -0.06 | 0.30 | 0.60 | 0.12 | 0.12 |
| *Lysimachia foemina* (Mill.) U.Manns & Anderb. | -0.05 | 0.16 | 0.66 | 0.00 | 0.14 |
| *Anthemis arvensis* L. | -0.06 | 0.27 | 0.58 | 0.12 | 0.12 |
| *Avena sterilis* L. | -0.12 | 0.33 | 0.55 | 0.15 | 0.12 |
| *Bromus sterilis* L. | -0.11 | 0.17 | 0.63 | 0.00 | 0.14 |
| *Chenopodium album* L. | -0.17 | 0.14 | 0.50 | 0.26 | 0.20 |
| *Cirsium arvense* (L.) Scop. | -0.12 | 1.00 | 0.55 | 0.24 | -0.13 |
| *Convolvulus arvensis* L. | -0.19 | 0.83 | 0.61 | 0.14 | 0.03 |
| *Crepis biennis* L. | -0.13 | 0.23 | 0.61 | 0.30 | 0.20 |

| Species | Provisioning | Regulating | Supporting | Cultural | FBI |
|---|---|---|---|---|---|
| *Cynodon dactylon* (L.) Pers. | -0.14 | 0.33 | 0.60 | 0.32 | 0.18 |
| *Dactylis glomerata* L. | -0.18 | 0.44 | 0.64 | 0.00 | 0.09 |
| *Daucus carota* L. | -0.12 | 0.34 | 0.60 | 0.40 | 0.19 |
| *Digitaria sanguinalis* (L.) Scop. | -0.08 | 0.25 | 0.65 | 0.00 | 0.12 |
| *Equisetum arvense* L. | -0.09 | 0.24 | 0.27 | 0.62 | 0.19 |
| *Euphorbia helioscopia* L. subsp. *heliscopia* | -0.08 | 0.35 | 0.67 | 0.14 | 0.13 |
| *Euphorbia prostrata* Aiton | -0.04 | 0.11 | 0.00 | 0.00 | -0.02 |
| *Fallopia convolvulus* (L.) Á.Löve | -0.14 | 0.25 | 0.68 | 0.00 | 0.14 |
| *Fumaria officinalis* L. | -0.08 | 0.31 | 0.60 | 0.00 | 0.09 |
| *Galium aparine* L. | -0.10 | 0.26 | 0.66 | 0.00 | 0.12 |
| *Helianthus annuus* L. subsp. *annuus* | -0.33 | 0.11 | 0.48 | 0.00 | 0.17 |
| *Helianthus tuberosus* L. | -0.35 | 0.25 | 0.47 | 0.00 | 0.14 |
| *Kickxia spuria* (L.) Dumort. | -0.05 | 0.09 | 0.00 | 0.00 | -0.01 |

| Species | Provisioning | Regulating | Supporting | Cultural | FBI |
|---|---|---|---|---|---|
| *Lactuca sativa* L. subsp. *serriola* (L.) Galasso, Banfi, Bartolucci & Ardenghi | -0.17 | 0.26 | 0.56 | 0.38 | 0.21 |
| *Legousia speculum-veneris*(L.) Chaix subsp.*speculum-veneris* | -0.03 | 0.16 | 0.66 | 0.12 | 0.16 |
| *Lolium multiflorum* Lam. | -0.13 | 0.21 | 0.58 | 0.00 | 0.12 |
| *Lolium perenne* L. | -0.11 | 0.39 | 0.56 | 0.00 | 0.07 |
| *Medicago lupulina* L. | -0.07 | 0.32 | 0.78 | 0.00 | 0.13 |
| *Medicago sativa* L. | -0.20 | 0.37 | 0.67 | 0.00 | 0.13 |
| *Trigonella officinalis* (L.) Coulot & Rabaute | -0.17 | 0.38 | 0.66 | 0.00 | 0.11 |
| *Mentha suaveolens* Ehrh. | -0.08 | 0.13 | 0.08 | 0.38 | 0.10 |
| *Nigella damascena* L. | -0.12 | 0.04 | 0.09 | 0.00 | 0.04 |
| *Ornithogalum umbellatum* L. | -0.07 | 0.17 | 0.05 | 0.00 | -0.01 |
| *Orobanche crenata* Forssk. | -0.08 | 0.16 | 0.00 | 0.00 | -0.02 |
| *Papaver rhoeas* L. subsp. *rhoeas* | -0.09 | 0.24 | 0.53 | 1.00 | 0.35 |

| Species | Provisioning | Regulating | Supporting | Cultural | FBI |
|---|---|---|---|---|---|
| *Helminthotheca echioides* (L.) Holub | -0.05 | 0.25 | 0.00 | 0.24 | 0.01 |
| *Picris hieracioides* L. | -0.13 | 0.42 | 0.60 | 0.30 | 0.15 |
| *Polygonum aviculare L.* subsp. *aviculare* | -0.08 | 0.21 | 0.57 | 0.24 | 0.17 |
| *Portulaca oleracea* L. | -0.06 | 0.42 | 0.53 | 0.14 | 0.08 |
| *Rapistrum rugosum* (L.) All. | -0.05 | 0.31 | 0.37 | 0.00 | 0.03 |
| *Rumex acetosa* L. subsp. *acetosa* | -0.08 | 0.33 | 0.64 | 0.32 | 0.18 |
| *Senecio vulgaris* L. | -0.00 | 0.20 | 0.00 | 0.37 | 0.04 |
| *Setaria verticillata* (L.) P.Beauv. | -0.08 | 0.16 | 0.00 | 0.00 | -0.02 |
| *Setaria italica* (L.) P.Beauv. subsp. *viridis* (L.) Thell. | -0.10 | 0.17 | 0.51 | 0.00 | 0.11 |
| *Sinapis arvensis* L. subsp. *arvensis* | -0.12 | 0.14 | 0.00 | 0.38 | 0.09 |
| *Sonchus asper* (L.) Hill | -0.11 | 0.19 | 0.51 | 0.12 | 0.14 |
| *Sonchus oleraceus* L. | -0.12 | 0.25 | 0.11 | 0.34 | 0.08 |
| *Sorghum halepense* (L.) Pers. | -0.26 | 0.34 | 0.62 | 0.00 | 0.13 |

| Species | Provisioning | Regulating | Supporting | Cultural | FBI |
|---|---|---|---|---|---|
| *Stachys annua* L. subsp. *annua* | -0.08 | 0.11 | 0.00 | 0.14 | 0.03 |
| *Trifolium pratense* L. | -0.11 | 0.24 | 0.76 | 0.24 | 0.22 |
| *Verbena officinalis* L. | -0.12 | 0.09 | 0.04 | 0.35 | 0.10 |
| *Veronica persica* Poir. | -0.06 | 0.24 | 0.70 | 0.00 | 0.13 |
| *Vicia sativa* L. | -0.17 | 0.16 | 0.56 | 0.00 | 0.14 |
| *Xanthium orientale* L. | -0.12 | 0.10 | 0.55 | 0.00 | 0.14 |
| *Xanthium spinosum* L. | -0.17 | 0.00 | 0.00 | 0.00 | 0.04 |

Legend: FBI, functional biodiversity index.

**Example of calculation procedure for functional biodiversity index at single species level.**

The objective of the present section is to supply an example of the calculation procedure of the functional biodiversity index, FBI, of a given species of the FunBies database. Such calculation procedure is equal for the whole set of species of the FunBies database.

Calculation procedure is based on a simplified version of Equation (3) in the manuscript text, where the species summation component was deleted. The modified equation is reported below.

$$FBI_{Sp} = \sum_{ES=1}^{4} * \left[ \sum_{EF=1}^{x} * \left( \sum_{FT=1}^{n} w_{FT} * A_{Sp} * S_{FT} \right) \right] \quad (4)$$

Where $W_{ES}$ is the weight attributed to each of the four ecosystem service categories, $W_{EF}$ is the weight attributed to each ecosystem function, $W_{FT}$ is the weight attributed to each functional trait, $A_{Sp}$ is the abundance of a species either in terms of number of individuals (nr/m$^2$) or of dry matter weight (g/m$^2$) $S_{FT}$ is the FT score per each species unit expressed either in terms of number of individuals or grams.

If we suppose ASp = 1, by sequentially aggregating FT scores at the levels of EFs and ES categories we obtain a functional biodiversity index (FBI) at species level that ranges between 0 and 1. It has to be noticed that this specific FBI represents the contribution that each species single unit can supply to functional biodiversity. Of course, the more abundant is a species in a field, in a hectare or in whatever reference area, the more it can contribute to overall functional biodiversity.

Table S38: Functional trait contributions to ecosystem functions (EFs), ecosystem services (ESs) and functional biodiversity index at specie level (FBIsp) calculated for *Cirsium arvense* L. Scop. For attribution of functional trait (FT) contribution to EFs) reference is made to Fig. S33. For the purpose of the example Asp = 1.

| Functional traits per ES category | $S_{FT}$ | $A_{Sp}$ | $w_{FT}$ | Trait contributions to EFs $w_{FT} * A_{Sp} * S_{FT}$ | $w_{EF}$ | Trait/EF contributions to ES categories $w_{FT} * A_{Sp} * S_{FT} * w_{EF}$ | $w_{ES}$ | Trait/ES contributions to $FBI_{Sp}$ $w_{FT}*A_{Sp}*S_{FT}*w_{EF}*w_{ES}$ |
|---|---|---|---|---|---|---|---|---|
| **Provisioning** | | | | | | | | |
| Plant biomass | -0.01 | 1 | 0.50 | 0.01 | 1.00 | -0.01 | 0.25 | 0.00 |
| Plant height generative | -0.34 | 1 | 0.20 | -0.07 | 1.00 | -0.07 | 0.25 | -0.02 |
| Seed weight | -0.15 | 1 | 0.09 | -0.01 | 1.00 | -0.01 | 0.25 | 0.00 |
| Drought tolerance | 0.00 | 1 | 0.08 | 0.00 | 1.00 | 0.00 | 0.25 | 0.00 |
| Nitrogen demand | -0.40 | 1 | 0.08 | -0.03 | 1.00 | -0.03 | 0.25 | -0.01 |
| Shade tolerance | -0.05 | 1 | 0.05 | 0.00 | 1.00 | 0.00 | 0.25 | 0.00 |
| **Overall Provisioning disservice** | | | | | | -0.12 | | -0.03 |
| **Regulating** | | | | | | | | |
| Root architecture | 1.00 | 1 | 0.50 | 0.50 | 0.17 | 0.09 | 0.25 | 0.02 |
| Canopy width | 0.39 | 1 | 0.25 | 0.10 | 0.17 | 0.02 | 0.25 | 0.00 |
| Drought tolerance | 0.00 | 1 | 0.25 | 0.00 | 0.17 | 0.00 | 0.25 | 0.00 |
| Root depth | 1.00 | 1 | 0.50 | 0.50 | 0.17 | 0.09 | 0.25 | 0.02 |
| Leaf dry matter content | 0.60 | 1 | 0.10 | 0.06 | 0.17 | 0.01 | 0.25 | 0.00 |
| Canopy width | 0.39 | 1 | 0.40 | 0.16 | 0.17 | 0.03 | 0.25 | 0.01 |
| Pollinator attractiveness | 0.55 | 1 | 1.00 | 0.55 | 0.17 | 0.09 | 0.25 | 0.02 |
| Alternative host/prey | 1.00 | 1 | 1.00 | 1.00 | 0.17 | 0.17 | 0.25 | 0.04 |
| Leaf C/N ratio | 0.57 | 1 | 1.00 | 0.57 | 0.17 | 0.10 | 0.25 | 0.02 |
| Plant tolerance to fire | 1.00 | 1 | 1.00 | 1.00 | 0.17 | 0.17 | 0.25 | 0.04 |
| **Overall Regulating ES provision** | | | | | | 0.75 | | 0.19 |
| **Supporting** | | | | | | | | |
| Leaf carbon content | 0.82 | 1 | 1.00 | 0.82 | 0.50 | 0.41 | 0.25 | 0.10 |
| Specific leaf area | 0.29 | 1 | 0.33 | 0.10 | 0.50 | 0.05 | 0.25 | 0.01 |
| Leaf C/N ratio | 0.57 | 1 | 0.33 | 0.19 | 0.50 | 0.09 | 0.25 | 0.02 |
| N-fixation | 0.00 | 1 | 0.33 | 0.00 | 0.50 | 0.00 | 0.25 | 0.00 |
| **Overall Supporting ES provision** | | | | | | 0.55 | | 0.14 |
| **Cultural** | 0.24 | 1 | 1.00 | 0.24 | 1.00 | 0.24 | 0.25 | 0.06 |
| **Overall Cultural ES provision** | | | | | | 0.24 | | |
| textbfFBI at species level | | | | | | | | 0.36 |

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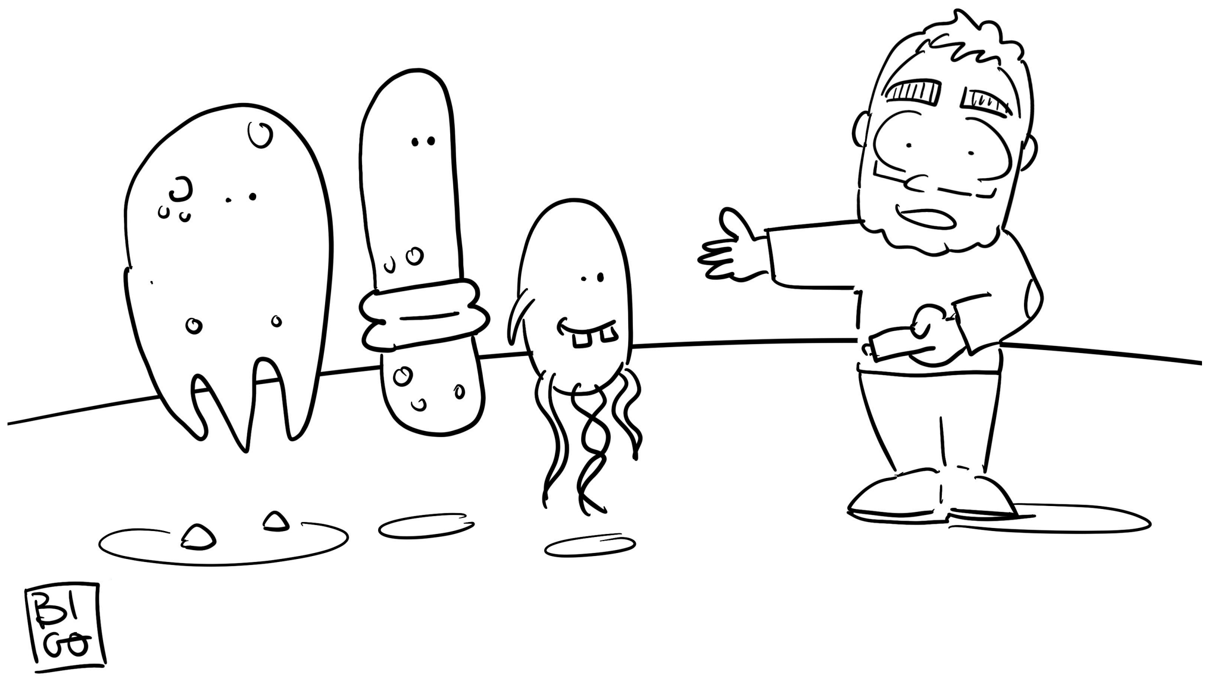
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