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Facing the Perfect Storm in the Mediterranean region: Can Organic Agriculture Play a Role by Enhancing Soil Fertility and Adaptation to Energy and Water crises?

Results From a 30-years Long-Term Experiment in Tuscany (Italy)

Academic Discipline (SSD) AGR/02

Doctoral Candidate
Dr. Santoni Margherita

(signature)

Supervisor
Prof. Pacini Gaio Cesare

(signature)

Coordinator
Prof. Viti Carlo

(signature)

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1 Abstract

The phrase "Perfect Storm" has been used to describe the future coincidence of food, water and energy insecurity. The current global energy crisis no longer allows the massive use of high energy inputs, such as chemical fertilizers, pesticides and irrigation. Several modelling studies have promoted the idea of organic farming being a viable option to face future adverse scenarios, mostly because of its capacity to achieve satisfying levels of food production while improving soil quality and consuming less resources. In the Mediterranean region, farmers have few technical and agronomical options due to arid conditions, prolonged droughts, scarce levels of water retention, most probably due to low levels of organic matter in soils.

Against this background, more insights are needed to enhance soil fertility by exploring alternative methods to high-input conventional agriculture. In this context, there is a compelling need to delve into agronomic practices that can reconnect crop and animal production, thereby enhancing soil chemical, physical, and biological fertility, with cascade effects on agroecosystems productivity and energy use efficiency.

The main objective of this Ph.D thesis was to carry out a systemic soil fertility assessment to asses organic and biodynamic agriculture as alternative methods to high-input agriculture in the Montepaldi Long Term Experiment (Italy), the most durable long-term experiment in the Mediterranean region where two arable farming systems — organic and conventional — have been running since 1992.

The results of the present thesis showed that yields significantly decreased with time in both organic and conventional systems (about -79% and -37% for spring and winter crops, respectively). This decrease could be attributed to a substantial drop (about -40%) in cumulative rainfall during the vegetative crop cycle and an increase in temperature (+1°C). Organic winter crops constantly yielded about 21% less than the conventional ones while spring crops did not show significant differences. Despite the higher productivity in conventional winter crops, the organic system showed a considerably higher energy use efficiency. For each unit of energy input, the energy output was found to be 33% higher in the organic system for winter crops. Even greater energy use efficiency was observed for spring crops, with a 44% higher efficiency in the organic. Therefore, the organic system undoubtedly exhibited better performance in terms of energy balance. In a country such as Italy, we can reasonably conclude that organic farming is an option to face the "Perfect Storm" in the Mediterranean, since it imports 2/3 of energy demand and cultivates only 12.5 million hectares of UAA as compared to 21.9 millions in the '60. Moreover, it was found that organically managed soils are more biologically active and less resistant to penetration, which might help farmers in storing more water and plants in reaching deeper layers in the soil profile. Such aspects of organic farming are promising but apparently they are not sufficient in coping with water scarcity. These problems require more advanced research on crop species and varieties more productive under water stress. The very same approach is required

for heterogeneous seed material having very diverse characteristics that allow it to evolve and adapt to growing conditions where water supply is restricted.

2 General Introduction

2.1 Background

The Mediterranean region stands at the forefront of environmental challenges, exacerbated by the impacts of climate change. Climate variations in the area are evident through the rising temperatures and the increasing frequency of extreme weather events, which are associated with looming scarcity of water resources (Lionello & Scarascia, 2018). The Mediterranean climate is undergoing rapid transformations, leading to increasingly noticeable impacts on ecosystems and human activities (Ali et al., 2022). This rapid transformation poses multifaceted challenges that affect agriculture, biodiversity, and socio-economic dynamics across the region.

The phrase "Perfect Storm" has been used to describe the future coincidence of food, water and energy insecurity (Godfray et al., 2010). Climate change 2022 impact report states that due to its particular combination of multiple strong climate hazards and high vulnerability, the Mediterranean region is a hotspot for highly interconnected climate risks. Climate change threatens water availability and yields of rainfed crops may decrease by 64% in some locations (high confidence), often due to increasing droughts (Ali et al., 2022). Increasing food production and water availability with high energy input requiring practices like fertilization with synthetic-chemical fertilizers and widespread use of irrigation does not seem to be a sustainable option when facing the current global energy crisis, ultimately defined as a shock of unprecedented breadth and complexity (IEA, 2022). The current global energy crisis no longer allows the massive use of high energy inputs, such as chemical fertilizers, pesticides and irrigation. Several modelling studies have promoted the idea of organic farming being a viable option to face future adverse scenarios, mostly because of its capacity to achieve satisfying levels of food production while improving soil quality and consuming less resources (Mäder et al., 2002; Muller et al., 2017; Poux & Aubert, 2018). However, further efforts are needed to understand to what extent organic agriculture can cope with adverse scenarios, given the different pedologic, climatic, and agronomic conditions.

Agroecosystems are characterized by a broad spectrum of interacting drivers that impact a potentially infinite number of components and processes, including functional biodiversity, energy flows, biogeochemical cycles, and interactions between organisms and biotopes. Considering these aspects, the ability to evaluate the impact of farming practices becomes overwhelmingly complex. To elucidate these intricate interactions, it is necessary to consider the results from specifically designed Long-Term Experiments (LTE), where the 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 searched within a sustainability choice space (Potschin-Young & Haines-Young, 2011) restrained by severe environmental and productive conditions, as is currently happening in the Mediterranean region. Here, farmers have few technical and agronomical options due to arid conditions, prolonged droughts, scarce levels of water retention, most probably due to low levels of organic matter in soils, often about 1.5% (Altobelli & Piazza, 2022).

Among all above-mentioned aspects of agroecosystems to be investigated, I chose to investigate soil chemical, physical and biological fertility due to its paramount importance with regards to organic matter flows, biogeochemical cycles and relevant impacts on agroecosystems productivity. Organic farming systems in the Mediterranean region are often stockless (Canali et al., 2005), even if the basic principles are based on the functional interconnection between crops and animal productions. Obviously, the stockless management eventually results in a scarcity of soil organic matter, which in turn is thought to be the main hurdle in coupling soil fertility with crop nutrition (Berry et al., 2002; Cormack et al., 2003; Stinner et al., 2008). Organic farmers were thus obliged to close the elements' cycles outside their farm, acquiring organic materials produced elsewhere: this externalization is a phenomenon which has been described as *conventionalization of organic farming* (Darnhofer et al., 2009).

In this context, biodynamic agriculture proposes an agroecological model which is based on a closed production system that includes livestock within the farm (Santoni, 2022). This model focused on reducing energy consumption, achieving high levels of environmental efficiency, and aiming for economic profitability (Bioreport, 2018). The controversy over biodynamic agriculture is often really a debate about science and spirituality. Some authors argue that the principles of biodynamics are scientifically untenable and unverifiable (Chalker-Scott, 2013), considering it as a pseudoscience (Parisi, 2021). On the countrary, other authors argue that biodynamic farming is compatible with pragmatic scientific approaches, and that its' a priori disqualification represents a missed opportunity for sustainability transformation (Rigolot & Quantin, 2022). In Italy, a recent bill proposal for acknowledging biodynamic farming as a suitable form of agriculture has generated a strong opposition and a petition by academic scientists (Ciliberto, 2022; Parisi, 2021). According to the petitioners, biodynamic farming cannot be verified through the scientific method, and the new law would amount to shaping government policy by esoteric astrological principles (Rigolot & Quantin, 2022).

In the current socio-cultural context where biodynamic farming is increasingly put to the fore in mainstream media, it seems necessary to investigate in a scientific context if biodynamic method could be a alternative solution for improving soil fertility in organic systems.

Organic farmers in the Mediterranean area maintain the fertility of their soils using organic amendments such as dried or pelleted manure, fresh manure, vermicompost, compost of food industry residues, etc. However, from a biological standpoint, biodynamic compost has been found to possess bio-active potential in the contexts of fertility and nutrient cycling (Giannattasio et al., 2013). Therefore, it seems necessary to investigate fertilization solutions that are able to reconnect crops and animal production, thus allowing the local unfolding of nutrient element cycles. Given the above described challenges, soil fertility is a major concern in agroecosystems management. Fertility is a complex and multifaced phenomenon, which requires a wide range of indicators to be tested and evaluated regarding the chemical, physical and biological soil properties. Soil fertility, defined by Mäder (2002) as the one that *provides essential nutrients for crop plant growth, supports a diverse and active biotic community, exhibits a typical soil structure and allows for an undisturbed decomposition*, is featured with long-term dynamics and needs to be assessed under a long-term perspective. Therefore, the analyses of this research project were carried out at the Montepaldi Long Term Experiment (MoLTE, San Casciano Val di Pesa, Florence, Tuscany[1], the most durable long-term experiment in the Mediterranean region where two arable farming systems — organic and conventional — have been running since 1992.

2.2 Problem Statement

Against this background, more insights are needed to enhance soil fertility by exploring alternative methods to high-input conventional agriculture. In this context, there is a compelling need to delve into agronomic practices that can reconnect crop and animal production, thereby enhancing soil chemical, physical, and biological fertility, with cascade effects on agroecosystems productivity and energy use efficiency.

2.3 Objectives of the Research

The main objective of this research was to carry out a systemic soil fertility assessment to asses organic and biodynamic agriculture as alternative methods to high-input agriculture in a long-term experiment in the Mediterranean region. To achieve this objective, tree phases were identified in the research project (Figure 1):

- To carry out a systemic soil fertility assessment through a wide range of indicators regarding chemical, physical and biological soil properties.
- To assess alternative agronomic techniques aimed at improving soil fertility through practices that reconnect crop and animal production, thereby allowing the local unfolding of nutrient element cycles.
- To provide a 30-year comprehensive analysis in a long-term experiment comparing organic and conventional agriculture, including climatic, agronomic, and soil parameters.

Phase 1, described in Chapters 3 and 4, entailed a systemic soil fertility assessment by comparing organic and conventional farming systems. In Phase 2, alternative fertilizing techniques aimed at improving soil fertility in organic systems were tested (Chapters 5 and 6). In Phase 3, an analysis of the data recorded over a 30-year period in the MoLTE field trial was conducted (Chapter 7).

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

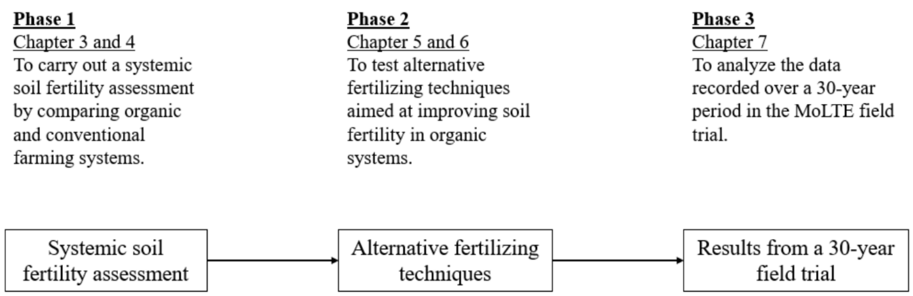

Figure 1: *Research outline.*

2.4 Outline of the Thesis

In Chapter 3 a soil fertility assessment of the impact of conventional and organic systems and conservation tillage on soil fertility at the MoLTE is carried out. A large set of indicators describing the state of soils in terms of chemical, physical and biological fertility was evaluated.

Chapter 4 focuses on microbial activity and soil quality in organic and conventional systems at the MoLTE. To assess soil fertility, the following indicators were used: bacterial and fungal biomass and activity, soil CO2 emission, and readily available nitrogen forms.

Chapter 5 assesses the state of the art of alternative forms of organic agriculture, such as biodynamic, whose agronomic techniques could enhance soil fertility. A review of international scientific literature on biodynamic agriculture was conducted to assess its performance.

Chapter 6 focuses on a three-year study conducted at MoLTE, investigating different types of organic fertilizers such as pelleted manure, fresh manure and biodynamic compost, which could improve soil fertility in organic systems.

Chapter 7 presents the results from a 30-year field trial at MoLTE, in which the agronomic performance of organic and conventional arable farming systems was compared. The MoLTE dataset, covering the period from 1993 to 2022, focuses on the main staple non-irrigated crops such as common and durum wheat, barley, maize, and sunflower. Moreover, it includes climatic variables (minimum and maximum daily temperature and rainfall), soil parameters, and agronomic records such as fertilizer amounts, tillage operations, sowing and harvesting dates, weeding, yields.

Chapter 8, i.e. Supplementary Materials Chapter, presents a model for integrated assessment of the functional biodiversity of weed communities in agroecosystems, denominated FunBies (i.e., FUNctional BIodiversity of agro-EcoSystems). The results of the FunBies application for the quantification of ecosystem services delivered by weed communities in organic and conventional systems at MoLTE are presented in this chapter.

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3 Assessment of the Impact of Conventional and Organic Agroecosystems Management Options and Conservation Tillage on Soil Fertility at the Montepaldi Long Term Experiment, Tuscany

Ottorino-Luca Pantani[1], Lorenzo Ferretti[1], Margherita Santoni[1], Simone Massenzio[1], Luigi Paolo D'Acqui[2], Gaio Cesare Pacini[1]

[1] Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali (DAGRI) - University of Florence (Italy)
[2] C.N.R., Istituto di Ricerca sugli Ecosistemi Terrestri, Via Madonna del Piano, 10 50019 Sesto Fiorentino, Firenze, Italy

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3.1 Abstract

Fertility is a characteristic of an agroecosystem which is usually and promptly identified with the crop yield. Nevertheless, it can be considered the result of many processes and factors such as climatic, edaphic and agronomic which cannot be extended and generalized to all systems and crops. This study evaluates the effects on soil fertility as influenced by organic (*OR*) and high-input (conventional, *CO*) management combined with three tillage systems, i.e., plowing (*plw*), chisel plowing (*chp*) and disk harrowing (*dsh*) at the Montepaldi Long Term Experiment (MoLTE), Tuscany, Italy. Fertility was evaluated through the following indicators: i) chemical (Olsen P, Kjeldahl N and, OM); ii) physical (bulk density on clods and cores, pore size distribution, penetrometry, aggregate stability, soil profile assessment, VESS, i.e. visual evaluation of soil structure); iii) biological (earthworm abundance and root distribution). As regards the effect of management, *CO* was higher in crop yields, available P_2O_5, bulk densities (clods), aggregate stability and soil penetration resistance, while *OR* was higher in bulk densities (cores). Nevertheless, the effect of management was observed for root distribution as a function of depth, where roots explored larger portions of soil in *OR* profiles. Regarding tillage, the order *plw, chp, dsh* was characterized by an increase in soil penetration resistance and number of earthworms. Moreover, a relationship with time was found for earthworm abundance, where the *OR* system exhibited a higher and constant population. Organic management seems to achieve a long-lasting soil fertility. In the MoLTE experiment results suggest that available P_2O_5, bulk density (clods), aggregate stability, soil penetration resistance, time-related earthworm abundance, root distribution and yields are the most informative on the impact of management and tillage options. Furthermore, results of physical and biological fertility indicators support the hypothesis that significant differences between *OR* and *CO* management, even if not observed in topsoil, might be detected in deeper soil layers, below 30 cm.

Keyword: soil health, soil quality, Mediterranean area, reduced tillage, compositional analysis, soil structure

3.2 Introduction

Soil fertility is a multi-faced aspect in agroecosystems management, both in terms of the broad range of properties defining it and for what concerns the drivers of land use. Among those drivers, both management options, say organic versus high-input, and tillage operations, say conservation or high intensity ones, may have a definite impact on soil fertility. Land use drivers, different combinations of chemical, physical and biological properties combined with highly heterogeneous parent material and climatic conditions, make the assessment of soil fertility a complex matter. Indeed, soil quality is more complex than the quality of air and water, not only because soil constitutes solid, liquid and gaseous phases, but also because soils can be used for a larger variety of purposes (Bünemann et al., 2018; Nortcliff, 2002).

In order to properly frame an assessment exercise on soil fertility, we first need to understand which are the specific targets of the assessment, i.e. those aspects of soil fertility that we consider of major importance. Under this perspective, it is useful to define soil fertility. In the literature there are a number of definitions. It is not an aim of this article to report all of them; rather, a vast range of definitions were reported and compared in Bünemann (2018), and semantic differences discussed in relation to terms such as "soil quality" and "soil health".

For the purpose of the present article we consider the definition of soil fertility given by Mäeder (2002) that define a fertile soil as the one that "provides essential nutrients for crop plant growth, supports a diverse and active biotic community, exhibits a typical soil structure and allows for an undisturbed decomposition". Among all definitions, this is the most similar to the concepts of soil quality and soil health. We chose it as it explicitly considers the whole set of chemical, biological and physical properties of fertility and it well describes soils capable of supporting biological systems that remain diverse and productive indefinitely, which is the implementation of the concept of sustainability according to the theory of Ecology.

The extent to which soil fertility is impacted by agroecosystems management options and tillage operations is assessed in this article as referred to typical conditions of inland hilly areas under the Mediterranean sub-Appenines climatic zone, which present semi-arid characteristics during the Spring-Summer season (Angeli et al., 2010).

Erosion, organic carbon loss and decline in biodiversity are the main challenges for areas with Mediterranean climate (FAO & ITPS, 2015). These phenomena are strongly interrelated as soil organic matter (OM) plays a major role in maintaining soil functions because of its influence on soil structure and stability, water retention and soil biodiversity, and because it is a source of plant nutrients. Indeed, some 45 % of soils in Europe have low or very low OM content (0-2 % organic carbon) and this is particularly evident in the soils of many southern European countries (FAO & ITPS, 2015).

On the other hand, the loss of OM in soils is due both to erosion and to the increased rate of mineralization of organic carbon in arable soils, which is due to intensive tillage operations, especially when combined with increased temperatures under climate change conditions. In inland hilly areas of Mediterranean Italy, where soils are often naturally susceptible to compression, such as in heavy textured soils, soil compaction is potentially an additional factor which inhibits the conservation and proliferation of OM due to decreased porosity, water retention capacity and to anoxic soil conditions.

In high external input farming, major threats of agricultural practices to soil biodiversity are due to soil contamination by pesticides, nitrogen and phosphorus fertilizers that cause negative impacts on efficiency and resilience of soil functionality, with glyphosate, the main herbicide used in Europe, detected in high concentrations in soils across the Mediterranean region (Ferreira et al., 2022; Silva et al., 2018).

Backed by these evidences on the agricultural origins of soil threats, there is increasing interest on the ability of organic farming practices to protect and foster soil fertility. It is often assumed that organic management performs better than conventional in terms of the capacity of soil systems to remain diverse and productive in the long-term (Mäeder et al., 2002).

Besides producing healthier food, avoiding pollution by chemicals and consuming less energy (European Parliament, 2016; Gomiero et al., 2008; Pimentel, 2006), this is the most positive advantage of managing agroecosystems with organic farming. Apart from specific cases, this benefit comes at the cost of a short-term decrease in land productivity as compared to high external input conventional agriculture (Ponisio et al., 2015). There appears to be a trade-off between temporary higher yields and the capacity to maintain soil productive and bio-diverse in the long-term.

Farmers can act on soil fertility not only by choosing different organic or high external input agroecosystems management options but also by applying conservation tillage practices. Under many pedo-climates, these practices showed to protect and improve soil fertility by decreasing erodibility and OM mineralization and by increasing soil cover, biodiversity, moisture retention and water infiltration rates (El-Hage Scialabba et al., 2014; Peigné et al., 2007).

However, many benefits of conservation tillage depend on how weed control is managed, as weeds are the major challenge of reduced and no-till systems (Holland, 2004). Different results can be expected from integrated pest management (IPM) treatments, genetically modified organisms (GMOs) coupled with glyphosate application or mechanical/manual weeding. Besides, the impacts of conservation tillage on yields can be highly variable depending on pedo-climatic characteristics, e.g. heavy soils combined with Mediterranean climates and zero or minimum tillage may cause crust formation and low rates of seedling emergence resulting in yield failures.

Backed by these considerations, the objective of this study was to investigate on the impact of two different agroecosystem management options, i.e. organic and high external input, and tillage operations (plowing, chisel plowing and disk harrowing) on soil fertility.

Fertility is a complex and multifaced phenomenon, which requires for a wide range of indicators to be tested and evaluated regarding chemical, physical and biological soil properties. Indicators should express the state of the soil as compared to threats (Bünemann et al., 2018). Besides, because visual soil assessment provides different information than laboratory approaches (Emmet-Booth et al., 2016) the combination of both would be advantageous (Bünemann et al., 2018; Pulido Moncada et al., 2014). We included in our analysis a large set of indicators describing the state of soils in terms of chemical, physical and biological fertility, the potential impacts in terms of soil erosion, compaction, conditions for supporting biological systems and increasing OM, and a combination of visual soil assessment and laboratory approaches.

The hypothesis at issue is that there is an urgent need to better understand how soil use and management impact soil fertility. This aspect is featured with long-term dynamics and needs to be assessed under a long-term perspective. We therefore carried out our analyses at the Montepaldi Long Term Experiment (MoLTE, San Casciano Valdipesa, Florence, Tuscany, https://www.dagri.unifi.it/vp-475-molte.html?newlang=eng), which is the longest experiment on organic farming of the whole Mediterranean area.

3.3 Materials and Methods

3.3.1 Site Description, Experimental Design and Sampling

The Montepaldi Long Term Experiment (MoLTE) has been active since 1991 at the experimental farm of the University of Florence (San Casciano Val di Pesa, Firenze, Tuscany, E 11°09'08'' N 43°40'16'', 90 m a.s.l.), covering a slightly sloping surface of about 15 ha. The soil of the experimental site is classified as Fluventic Xerochrepts and is between silty clay loam and clay loam in terms of texture (Migliorini et al., 2014). Three stockless arable systems are maintained: i) a conventional/high-input one[2], since 1991, ii) an organic one (EC reg. 2092/91 and following regulations) since 1992 and iii) an integrated one (EC regulations 2078/92) until 2001, which was then converted to organic. Natural and artificial hedges are interposed between the three agroecosystems, to reduce the risk of interactions and cross-contaminations (Migliorini et al., 2014). In i) chemical xenobiotics, mineral and synthetic fertilizers have been applied since 1991, while in ii) and iii) organic-certified mineral fertilizers, amendments and green manure were used from 1991 until 2013, when the OM restoration ended due to the shift of research objectives to tillage operations, as described below. The experiment under discussion here only considers i) and ii), where two factors were evaluated: management (MAN) with two levels — Conventional (CO) and Organic (OR) — and tillage (TIL), with three levels: plowing, *plw*, chisel plowing, *chp*, and disk harrowing, *dsh*. The above described primary tillage operations, sorted for intensity were repeatedly performed on the same plot three times from year 2015 to year 2017 (Figure 7).

The agronomic aspects of the experiment are described in Table 1. Based on the location of the main crop (barley and sunflower) in the rotation, two fields ($FIELD$, 47 x 132 m each) per management option (OR01, OR03, CO09

[2]From now on these two words will indicate the very same management.

and $CO10$ in the 2015/2016 campaign and $OR02$, $OR04$, $CO09$ and $CO10$ in 2016/2017 campaign) were divided into 9 plots, 12 x 36 m each) where three replicates (REP) for each tillage option were allocated (Figure 7). Within each plot, three sampling schemes were used (Table 5);

linear (LIN): three sampling sites were identified within each plot, one in the center (m) and two others 4 m to its left (l) and to its right (h), along the main axis of the plot;

triangular (TRI): three sampling sites were roughly located at the vertices of an equilateral triangle with its centre in site m;

profiles (PRO): six profiles, (1.5 m deep, 2.1 m wide, 1.5 m large) — one for each $MAN*TIL$ combination — were excavated in $OR02$ and $CO10$.

Table 5 reports the chronology of data collection as well as which sampling scheme was used for each indicator. The sampling details are described in the relevant section below.

Table 1: *Agronomical details of the MoLTE experiment from 2015 to 2017. The abbreviations* OR *and* CO *indicate organic and conventional managed fields, while 1, 2, 3, 4, 9,10 indicate the number of a single field.*

	2015/2016				2016/2017			
Field	OR01	OR03	CO09	CO10	OR02	OR04	CO09	CO10
Previous crop	*Cicer arietinum*, var. Pascià	*Trifolium alexandrinum*, var. Alex	*Hordeum vulgare*, var. Campagne	*Heliantus annuus*, var. Solaris[i]	*Lens culinaris*, var. Val di Nevola	*Cicer arietinum*, var. Pascià	*Heliantus annuus*, var. Solaris[i]	*Hordeum vulgare*, var. Sidney[l]
Actual crop	*Hordeum vulgare*, var. Sidney	*Helianthus annuus*, var. Solaris	*Helianthus annuus*, var. Solaris[i]	*Hordeum vulgare*, var. Sidney[l]	*Heliantus annuus*, var. Solaris	*Hordeum vulgare*, var. Campagne	*Hordeum vulgare*, var. Campagne[l]	*Helianthus annuus*, var. Solaris [i]
Plant density	190 kg ha^{-1}	4.5 kg ha^{-1}	4.5 kg ha^{-1}	190 kg ha^{-1}	4.5 kg ha^{-1}	190 kg ha^{-1}	190 kg ha^{-1}	4.5 kg ha^{-1}
Primary tillage[a]	Sep/07/2015	Sep/07/2015	Sep/07/2015	Sep/07/2015	Sep/08/2016	Sep/08/2016	Sep/08/2016	Sep/08/2016
Disk harrowing	Nov/09/2015	Mar/15/2016	Mar/15/2016	Nov/09/2015	Feb/23/2017	Dec/05/2016	Dec/05/2016	Feb/23/2017
Harrowing	-	Apr/04/2016	Apr/04/2016	-	Mar/29/2017	-	-	Mar/29/2017
Pre-sowing fertilization	-	-	-	Nov/08/2015[b]	-	-	Dec/05/2016[b]	-
Sowing	Nov/09/2015	Apr/04/2016	Apr/04/2016	Nov/09/2015	Mar/30/2017	Dec/05/2016	Dec/05/2016	Mar/30/2017
First fertilization	-	-	Apr/04/2016[g]	Mar/14/2016[c]	-	-	Mar/15/2017[c]	Mar/30/2017[g]
Chemical hoeing	-	-	Apr/04/2016[e]	Apr/01/2016[d]	-	-	Mar/29/2017[d]	Mar/30/2017[e]
Weed hoeing	-	May/26/2016	May/26/2016	-	May/31/2017	Mar/15/2017	-	Not executed[h]
Second fertilization	-	-	May/26/2016[f]	Apr/04/2016[f]	-	-	Apr/11/2017[f]	Not executed[h]
Harvest	Jun/29/2016	Sep/05/2016	Sep/05/2016	Jun/29/2016	Aug/24/2017	Jul/07/2017	Jul/07/2017	Aug/24/2017

[a] Plowing, disk harrowing and chisel plowing, based on the experimental design
[b] $(NH_4)_2HPO_4$ 192 kg ha^{-1}
[c] NH_4NO_3, 150 kg ha^{-1}
[d] Axial (1 L ha^{-1}) (a.i. pinoxaden 10.6 % and cloquintocet-mexyl 2.55 %) + Axial Pronto (0.75 L ha^{-1}) (a.i. pinoxaden 6,4 % and cloquintocet-mexyl 1.55 %) + Logran (37 g ha^{-1}) (a.i. triasulfuron 20 %)
[e] GOAL 480 SC 0.5 L ha^{-1} P.a. oxifluorfen
[f] urea150 kg ha^{-1}
[g] 20.10.10150 kg ha^{-1}
[h] due to both excessive presence of weeds and missing sunflowers
[i] seeds treated with Apron-xl a.i. metalaxil-m 30.95%
[l] seeds treated with Redigo, a.i. propiconazole 8.7 %

3.3.2 Chemical and Physical Indicators

3.3.2.1 Available P_2O_5, Total N and OM The soils were sampled during the spade test (Table 2); for each sample and layer identified through the spade test, the following chemical indicators were measured: available P_2O_5 (Olsen et al., 1954), total N content (Kjeldahl, 1883) and OM (Walkley & Black, 1934).

3.3.2.2 Bulk density: Core and Clod Methods The plots were sampled two months after the primary tillage for all the fields and sampling sites (Table 5) by means of a brass cylinder (9.5 cm diameter, 12 cm height) inserted into the soil. The soil core was sealed in a plastic bag, brought to the lab, suspended in water and passed through a 2 mm sieve (Ugolini & Certini, 2010). The volume of the coarser fraction (V_{Ske}) was measured by hydro-static buoyancy in water and subtracted from the sampled volume (V_{Cyl}). The finer fraction was dried to constant mass at 105 °C and weighed (P_{105}). In 2016 all the samples (108) were measured for bulk density while in 2017 only the m samples of the linear scheme were measured (36). The above-described indicator will be referred to as *Core* bulk density. The bulk density ρ_{Core} was calculated by

$$\rho_{Core} = \frac{P_{105}}{V_{Cyl} - V_{Ske}} \tag{1}$$

In the 2017 sampling session (Table 5), a shovel of soil was taken within the first 20 cm, sealed in a plastic bag and brought to the lab, where three aggregates of centimetric size for each bag, randomly chosen, were immediately analyzed for bulk density with hydro-static buoyancy as described by Monnier (1973): briefly, the aggregates (3–4 cm diameter) were kept under petroleum (d = 0.761 g cm^{-3}), the excess petroleum removed, the buoyancy B_{tot} measured ($\pm 10^{-3}$ g sensitivity), the aggregate dried at 150 °C, weighed (P_{150}) and the bulk density ρ_{Clod} calculated by:

$$\rho_{Clod} = \frac{P_{150}}{\frac{B_{tot}}{0.761}} \tag{2}$$

A total of 324 measurements were performed. The above described measure will be referred to as the *Clod* method.

Core and *Clod* methods were selected for two different reasons i) to give insights on soil structure in two different domain ii) to have data from a simple, yet informative method, as well as from a much complicate one.

3.3.2.3 Total Porosity Total porosity[3] was measured on air dried aggregates about 2.5 cm in diameter by mercury intrusion (Carlo Erba, Porosimeter 2000) in the 0.007–200 µm equivalent cylindrical diameter (ECD) range, which confidently includes the micro-, meso-, and the lowest range of macro-porosity of the soil (less than 0.5 µm, between 0.5 µm and 50 µm, and greater than 50 µm

[3] In the present work the term *total porosity* indicates the pores detectable by Hg intrusion technique.

respectively). The surface tension of mercury and its contact angle on the sample were 0.480 N m^{-1} and 141.3°, respectively. Samples were taken from *m* site, 18 aggregates were measured, three replicates for each *MAN*TIL* combination. The replicates were randomly withdrawn from each *FIELD*. The total porosity (mm^3 g^{-1}) was calculated from the area under the distribution.

3.3.2.4 Soil Penetration Resistance The penetrometry measurement sessions (0–80 cm) were performed on three subsequent days in Autumn 2015 and 2016 with an Eijkelkamp Penetrologger. On each day, 12 plots out of 36 - one for each *TIL* and *FIELD* - were tested and the measurements were taken at the *l, m, h* sites in each plot. A total of 108 measures were performed each year.

3.3.2.5 Aggregate Stability The analysis of soil aggregate stability in water was performed on samples which were dried at 105 °C. In order to obtain insight into slaking — the aggregate breakdown due to internal stresses caused by rapid water uptake that compresses air — 300 mg aliquots of calibrated aggregates (0.5-1 mm) both dry and pre-wetted by gently spraying deionised water were immersed in distilled water circulating in a wet sample dispersion unit of a laser granulometer analyzer (Malvern Mastersizer 2000). The fragment/particle size distribution of suspended material was recorded after each minute for 12 min. After this time an ultrasonic transducer was activated (max. power 35 W) and the fragment/particle size distribution of suspended material was recorded every each minute until the particle size distribution of dispersed particles was constant (around 24 min). The median diameter (equivalent diameter d_{50}) of the particle-size distribution, interpolated with a logarithmic function, was assumed as an estimate of soil aggregates stability (Table 2). The entire dataset (changes in particle size distribution over time) was also analyzed compositionally as described in the data analysis section. A total of 36 *Dry* + 36 *Wet* samples (collected in each *m* point), corresponding to the combination of the factors and the level of the experiment (2 *MAN* * 3 *TIL* * 2 *FIELDS* * 3 *PLOT*) were analyzed.

3.3.3 Biological Indicators

3.3.3.0.1 Earthworm Abundance According to the VESS method (B. Ball et al., 2007), earthworms were hand-sorted within a soil cubic block (25 cm side) and then counted. Earthworms were considered only as number of individuals, while information on age, species, size, ecotype, etc. were not considered. From an ecological point of view we point out that the population was entirely composed of anecic earthworms (Paoletti et al., 2013) from the *Hormogaster* genus as established by genome sequencing (data not shown).

3.3.3.0.2 Root Distribution According to the *Grid method* developed by Tardieu and Manichon (1986), roots were counted within each of the six soil profiles (sampling scheme PRO) by using a plastic net (1 m long, 0.7 m wide, square holes 2 cm side) pinned on the soil profile. The number of roots for each square hole were recorded, then the plastic net was moved to the right and the counting procedure repeated until the profile width was covered. Each root system was therefore mapped with a resolution of 4 cm^2 (Figure 4).

3.3.4 Visual Indicators

3.3.4.0.1 Spade Test Soil structure was evaluated with a spade test, in accordance with the VESS method (B. Ball et al., 2007; Vian et al., 2009). Root observation and macropore counting was developed by Joséphine Peigne and Jean-Francois Vian (ISARA Lyon, http://www.fertilcrop.net/fc-publications/technical-notes.html). Table 5 reports sampling date and sampling scheme for each spade test diagnosis. The evaluation takes into account five steps:

(i) the cutting out of a spade-sized soil block leaving one side undisturbed. Therefore, length of the soil block is measured. At this stage, the undisturbed side of the block is opened like a book to be analysed;

(ii) the identification of distinct layers of differing structure, if any. For each soil layer, the degree of firmness and the size of soil fragments clods and aggregates (clods are defined as large, hard, cohesive and rounded aggregates, larger than 7 cm) are observed. If the block is uniform it must be assessed as a whole;

(iii) the breaking up of the soil into smaller structural units from 1.5 to 2 cm to assess shape, porosity and evidence of anaerobism (colour, mottles and smell) for each identified soil layer;

(iv) the observation of crop rooting in order to identify clustering, thickening, defections, distribution, if any;

(v) the estimation of the presence of earthworm macropores through counting burrows;

In accordance with the VESS method standard (Figure 11), a score from 1 (good structure) to 5 (poor structure) based on the previous observations is assigned to each soil layer and then a weighted mean is calculated in order to obtain a soil block score.

3.3.4.0.2 Soil Profile Assessment The soil profile assessment (Boizard et al., 2017) was aimed at investigating the effects of *MAN* and *TIL* on both structure and agronomical functionality of the soil in the surface, deep and transition layers. The soil condition diagnosis was made via the use of synoptic tables. Based on the PRO sampling scheme, the assessment was performed as follow:

1. To better identify the various colours of the soil, the lightest side was chosen and the surface refreshed with a knife before the observations began;

2. Different layers due to different tillage (past and recent), compaction and change in texture were detected. At this step, tillage pan and wheel tracks can be observed;

3. Clods > 2 cm were classified according to the proportion of structural porosity visible (Peigné et al. 2018) : (1) clods with a loose structure exhibit a clearly visible structural porosity and are called gamma Γ clods; (2) clods with few biological macropores (earthworms, roots) visible on a smooth face correspond to moderately compacted clods: these are called Δb clods; and (3) clods with no visible structural porosity and evidence of severe compaction, are called delta Δ clods;

iv) Humidity, earthworm burrows and casts, portion of soil explored by the roots and change in colors due to reduction and oxidation were observed. These observations were made in the 0–40 cm soil layer i.e. the portion occupied by the crop roots.

3.3.4.1 Yield For each *PLOT*, three sampling sites with random coordinates $(x_1, y_1, x_2, y_2, x_3, y_3)$ where identified in the field. On each x_n, y_n site, a squared frame ($0.25\,\mathrm{m}^2$) was used to collect barley plants, while a two meters long ruler was used to select sunflowers row-wise. Dry matter yield was then calculated by averaging the three x_n, y_n samples and eventually by standardizing barley grains and sunflower seeds to ton ha^{-1}.

3.3.5 Statistical Analysis and Data Treatment

The analytical process was as follows;

(i) to provide an overall summary of the data, the indicators were analyzed and ANOVA followed by a HSD Tukey test were performed, except for number of earthworms, number of roots and score of the spade test since those data showed deviation from normality.

(ii) root number and earthworm abundance were treated as counts and analysed with Generalized Linear Models (GLM), with a Poisson distribution and a log link function; data from spade test were not normally distributed (Kruskall-Wallis test p = 0.001) and therefore the differences were investigated through a Wilcoxon pairwise comparison; data from aggregate stability were considered as compositional, *sensu* Aitchison [-@aitchison1986statistical].

(iii) For each data class in i) and ii), comparison of marginal models was used in order to find the simplest model — the one with the least number of significant descriptors — capable of describing the data variability. For data class in i), ANOVA was performed on the final model for each indicator and analysis of residuals did not show substantial deviation from normality.

All analyses were performed using the R statistical software version 4.3.2 (R Core Team, 2020) and some of its libraries (Dahl, 2016; De Mendiburu, 2016; Lê et al., 2008; Sarkar, 2008; van den Boogaart et al., 2014; Wickham, 2009, 2011). Linear and generalized linear models were built by lm() and glm() functions. The dropterm() and stepAIC() functions (Venables & Ripley, 2002) were used to explore the model space for *lm* and *glm* R classes, while for *acomp* classes the exploration of model space was performed manually, following the indications of den Boogaart (2013). The procedures of reproducible research were accomplished by Sweave (Leisch, 2002) and version control by Git (VV.AA., 2022).

Table 2: *Mean values of the indicators measured in the experiment: different letters represent significant means within row after a Tukey test (q=0.95). Numbers between parentheses are the number of samples considered.*

	Conventional						Organic					
	Plowing		Chisel plowing		Disk harrowing		Plowing		Chisel plowing		Disk harrowing	
Parameter	2015/2016	2016/2017	2015/2016	2016/2017	2015/2016	2016/2017	2015/2016	2016/2017	2015/2016	2016/2017	2015/2016	2016/2017
P_2O_5, mg kg^{-1}	27.49ab(3)	25.02abc(3)	28.40ab(3)	32.72a(5)	27.85ab(6)	26.58ab(6)	11.75bc(4)	4.86c(6)	12.83bc(5)	6.71bc(5)	15.07abc(6)	10.98bc(6)
OM %	2.64a(3)	2.63a(3)	2.95a(3)	2.69a(5)	3.01a(6)	2.65a(6)	2.48a(4)	2.65a(6)	2.64a(5)	2.75a(5)	2.70a(6)	2.75a(6)
N, g kg^{-1}	1.12a(3)	1.09a(3)	1.16a(3)	1.13a(5)	1.21a(6)	1.12a(6)	1.06a(4)	1.12a(6)	1.13a(5)	1.13a(5)	1.13a(6)	1.13a(6)
BD Core, g cm^{-3} [a]	1.34a(6)	1.38a(6)	1.38a(6)	1.26a(5)	1.35a(6)	1.35a(6)	1.42a(6)	1.37a(6)	1.42a(6)	1.40a(6)	1.41a(6)	1.35a(6)
BD Clod, g cm^{-3} [b]	-	1.93a(18)	-	1.90ab(18)	-	1.89ab(18)	-	1.90ab(18)	-	1.87ab(18)	-	1.84b(18)
Penetr., $\log_{10}$(MPa)	0.14bcd(18)	-0.04f(18)	0.18abc(18)	0.02ef(18)	0.28a(18)	0.12cde(18)	0.10cde(18)	-0.04f(18)	0.12cde(18)	0.03ef(18)	0.24ab(18)	0.05def(18)
Penetr., MPa	1.39	0.91	1.52	1.05	1.91	1.33	1.25	0.92	1.33	1.06	1.73	1.11
Tot. porosity, mm^3 g^{-1}	-	171a(3)	-	168a(3)	-	186a(3)	-	157a(3)	-	174a(3)	-	196a(3)
Diam. aggr., µm [c]	-	239a(6)	-	216a(6)	-	206a(6)	-	163a(6)	-	214a(6)	-	189a(6)
Spade test [cd]	1.00a(18)	1.06ab(18)	1.06ab(18)	2.28ab(18)	2.17ab(18)	1.50ab(18)	1.11ab(18)	1.39ab(18)	1.61ab(18)	1.83ab(18)	2.22b(18)	1.61ab(18)
N. of earthworms [e]	0.17 (12)	0.50 (12)	1.50 (12)	2.92 (12)	2.58 (12)	6.33 (12)	1.08 (12)	0.58 (12)	5.08 (12)	3.25 (12)	5.67 (12)	5.00 (12)
Root number, m^{-2} [ef]	3113 (3)	-	2548 (3)	-	2994 (3)	-	3523 (3)	-	3660 (3)	-	3201 (3)	-
Barley, ton ha^{-1}	5.02a(3)	4.47ab(3)	4.96a(3)	4.49ab(3)	4.96a(3)	3.94ab(3)	3.65abc(3)	2.94bc(3)	3.31bc(3)	2.31c(3)	3.25bc(3)	2.18c(3)
Sunflower, ton ha^{-1}.	4.52a(3)	0.17c(3)	3.35ab(3)	0.17c(3)	2.68abc(3)	0.40c(3)	2.45abc(3)	1.40bc(3)	2.94abc(3)	1.00bc(3)	1.58bc(3)	1.13bc(3)

[a] Bulk density measured with the *Core* method; [b] Bulk density measured with the *Clod* method;
[c] Spade tests for sunflower fields in 2016-2017 and weighed mean for aggregate stability for wet conditions are not considered;
[d] Non-normal data: Wilcoxon pairwise comparisons (Bonferroni's method adjusted) after a Kruskal-Wallis test at $p < 10^{-3}$ was performed;
[e] The serious departure from normality did not allowed to perform the Tukey test. A detailed analysis was necessary and is reported in subsection 3.4;
[f] Root density was recorded in the field in 5250 squares, 4 cm^2 each. Above, roots$*$ m^{-2} are reported since the original counts gave an exceedingly high number of degrees of freedom

3.4 Results

The overall results of the descriptive statistical analysis are shown in Table 2. The result for each group of indicators (chemical, physical, biological and visual) are reported below.

3.4.1 Chemical and Physical Indicators

3.4.1.1 Chemical Indicators Available P_2O_5 was significantly higher in the *CO* system, while significant differences in total N and OM% were not found. No significant differences were found between tillages.

3.4.1.2 Bulk Density The results obtained through the *Core* method are summarized in Figure 8.

The ANOVA (Table 7) indicates the non-significance ($p >= 0.05$) for all the considered experimental factors except for *MAN*, which is slightly below the 0.05 critical value. The general mean was $1.37\,\mathrm{g\,cm^{-3}}$, and it is similar to the values commonly observed in soils. The mean values for *CO* and *OR* soils were 1.34 and $1.40\,\mathrm{g\,cm^{-3}}$, respectively, thus indicating a slightly more compacted soil for *OR* fields

For *Clod* method the results are summarized in Figure 9 and Table 8. The mean bulk density ($1.89\,\mathrm{g\,cm^{-3}}$) is higher than the one measured by the *Core* method.

Table 8 shows that both *MAN* and *TIL* are significant ($p < 0.05$). The Tukey test (Table 2) shows that there is a central homogeneous group, beside which the *CO-plw* and *OR-dsh* fields show the higher and the lower density, respectively. Nevertheless, it should be noted that the differences are in the range of the centesimal figure, i.e. a value with no practical consequences, the significance being due to the high number of clods examined (324).

3.4.1.3 Soil Penetration Resistance Table 11 shows the ANOVA table, and shows that the soil penetration resistance is significantly influenced by all the factors considered in the experiment. The resistance values (MPa) were log transformed to fulfil the ANOVA assumptions. The penetrometry data (mean values, depth 0–80 cm) is also summarized in Figure 1. In 2015/2016 significant lower soil penetration resistance were observed for *plw* and *chp* in *OR* system compared to the same tillage in *CO* system.

3.4.1.4 Aggregate Stability The stability of aggregates in soil was compositionally analyzed, *sensu* Aitchison (1986), since no evidence arose from a customary ANOVA analysis (Table 2). The exploration of model space through comparison of many marginal compositional models (Boogaart et al., 2013), allowed us to establish that i) the composition of suspended fractions is quadratically linked to time and ii) *MAN* has significant effects while *TIL* does not (ANOVA in Table 12). The aggregate's breakdown — ternary composition of size of material in suspension — as a function of time is shown in Figure 2: the

Table 3: *Results for penetrometry. ANOVA assumptions were fulfilled by log-transforming raw data. The first column reports back-transformed data in MPa*

	MPa	$\log_{10}$(MPa)	Std. Error	t value	Pr(>|t|)
CO plw 15/16	1.38	0.140	0.010	9.480	$< 10^{-3}$
YEAR 16/17	-0.40	-0.150	0.010	-11.970	$< 10^{-3}$
MAN Or	-0.12	-0.040	0.010	-2.840	$< 10^{-3}$
TIL chp	0.17	0.050	0.020	2.990	$< 10^{-3}$
TIL dsh	0.48	0.130	0.020	8.300	$< 10^{-3}$

colored dots are snapshots of the suspended material. On the leftmost side of the cloud of dots is visible a series of blue aligned points, produced by a single sample, one *dot/frame* taken from zero to minute 23. So, as the time pass by, the composition of suspended particles moves from a coarser composition to a finer one. Solid lines indicate the quadratic relationships between the composition and time (model reported in Table 6).

The effect of slaking is evident from the difference in composition between *Wet* and *Dry* samples (Table 12), these last ones being able to produce lower percentages of particles greater than 250 µm at the start of the measure, when the explosive power of trapped air is at its maximum (Figure 2). The initial composition is influenced by *MAN*, while the evolution along time is not. Both in *Dry* and *Wet* conditions, the *CO* fields produced coarser particles than *OR* ones at the beginning of the disgregation.

3.4.2 Biological Indicators

3.4.2.1 Earthworm Abundance Earthworm data was treated through a time regression based on the sampling in order to better define the earthworm population dynamic through the seasons. As we can see in Figure 3, earthworm abundance is generally higher in the *OR* system (except than in *CO-dsh*) and the number of earthworms increases from *plw* to *dsh*. Furthermore, in the *OR* system, earthworm abundance was constant, while it increased from November 2015 to March 2017 in the *CO* system.

Table 4: *Summary of the expected number of earthworms as estimated by GLM at the beginning (start) and at the end (end) of the experiment.*
Odd rows contain estimates of the values and the probability of being different from zero, even rows contain the difference against the row immediately above. The first column reports back-transformed data in expected number of earthworms as from the formula $earthworm\,n = e^{Estimate}$.

	n.of.ea.worms	Estimate	Std. Error	z value	Pr(>|z|)
start CO plw	0.165	-1.801	0.382	-4.718	$< 10^{-3}$
start OR plw	0.664	1.614	0.450	3.589	$< 10^{-3}$
start CO chp	1.094	0.090	0.199	0.452	0.652
start OR chp	3.052	1.332	0.237	5.626	$< 10^{-3}$
start CO dsh	2.209	0.792	0.173	4.571	$< 10^{-3}$
start OR dsh	3.098	0.877	0.211	4.162	$< 10^{-3}$
end CO plw	0.542	-0.612	0.360	-1.700	0.089
end OR plw	0.296	0.435	0.431	1.009	0.313
end CO chp	3.591	1.278	0.154	8.319	$< 10^{-3}$
end OR chp	0.597	0.154	0.200	0.769	0.442
end CO dsh	7.250	1.981	0.119	16.687	$< 10^{-3}$
end OR dsh	-1.889	-0.302	0.168	-1.795	0.073

Table 4 report the expected number of earthworms as estimated by GLM (Table 6) at the beginning and at the end of the experiment for each *MAN*TIL* combination. At the beginning of the experiment (rows 1-6) the expected number of earthworms was significantly higher in *OR* system compared to *CO* system for each tillage (difference between odd and even rows is always positive). Although the differences were not significant, the same behaviour can be observed at the end of the experiment (rows 7-12) apart from *dsh* in which the expected number of earthworms in *OR* system was lower than the one in *CO* system (negative difference between rows 11 ad 12).

3.4.2.2 Root Distribution Figure 4 shows the collected data for the six soil profiles excavated in May 2016. A GLM was applied to the data, and the results are shown in Figure 5. The formal analysis and ANOVA tables are reported in Table 15 and Table 16.

The root distribution depicted in Figure 4 and described in Figure 5 indicates two major features:

(i) *OR-chp* profile is the richest in roots in the first 20 cm, reaching a value at about 1.25 roots per cm^2;

(ii) *OR-plw*, albeit less dense in the shallow layers, shows a slower decay of roots density along the profile.

Figure 5 show that, at depth of 1 cm the expected number of roots per $4cm^2$ are 5.0, 4.3, 4.1, 3.8, 3.6, 3.5 for *Or-chp, Or-dsh, Co-plw, Co-dsh, Co-chp, Or-plw* , respectively.

As it concerns the slope, taking *CO-plw* (black solid line) as reference, there is not significant difference between *CO-dsh* (light grey solid line) and the reference (Table 15, row 8). On the contrary, there is a significant difference between the reference and the rest of the *MAN*TIL *combinations. Furthermore, the expected root number trend (see Table 15, rows 6, 7, 9, 10) in* OR-plw *(black dotted line) is the most striking aspect to emerge from; in the very first soil layers, the expected root number is lower than in the other* MAN*TIL* combinations, but it decreases more slowly along the profile (the steeper the slope, the slower the expected decrease in root number).

3.4.3 Visual Indicators

3.4.3.1 Spade Test No differences between *MAN* and *TIL* were identified, except for *CO-plw* and *OR-dsh* in 2015/16 (Figure 10). An improved gradient could be observed from reduced (*chp, dsh*) to ordinary tillage (*plw*) in 2015/16 and in 2016/17 in *OR* system. Furthermore, *YEAR* slightly affect the score assigned to the soil samples. Overall, soil resulted more compacted in 2016/17 than in 2015/16. A score of 2 was assigned in 68 and 57 % of the cases in *CO* and *OR* systems respectively, a score of 3 was assigned in 27 and 42 % of the cases in *CO* and organic systems respectively, and a score of 4 was assigned three times in the *CO* system and one time in the *OR* system.

3.4.3.2 Soil Profile Assessment No statistical analysis was performed on the soil profile assessment and the results of the observation referring to the soil structure are shown in Figure 6.

In the 0–15 cm soil layer the percentage of porous zones and compacted zones with presence of biological activity ($\Gamma + \Delta_b$ clods), was higher in the *OR* system for *plw* and *chp* with 92.5% and 90% respectively compared to the 85% and 70% observed in the *CO* system. In contrast, disk-harrowed soil showed 100% of porous zones (Γ clods) in the *CO* system compared to the 70% recorded in the *OR* system. In the 15–40 cm soil layer the percentage of porous zones and compacted zones with presence of biological activity was higher in the *OR* system for each tillage, with 85%, 95% and 85% respectively for *plw*, *chp* and *dsh* compared to the 80%, 75% and 40% observed in the *CO* system. Furthermore, *chp* soil showed the highest percentage of Γ clods. As regards compaction, humidity, earthworms and root activity along the profile (0–40 cm), the principal results were:

(i) plowed soil showed higher humidity in the *OR* than in the *CO* system. Also, a plow pan at 35 cm depth was observed in both the *OR* and *CO* systems;

(ii) chisel-plowed soil was generally drier and harder in the *CO* than in the *OR* system;

(iii) the undisturbed soil in the 15–40 cm soil layer was more compacted under *dsh* compared to *plw* and *chp*, but a higher activity of macro-organisms, such as earthworms, was observed;

(iv) for each tillage, roots were widely distributed along the whole profile in the *OR* system, while they featured only in the superficial layers in the *CO* system.

3.4.3.3 Yield As it regards management, yield was greater in the *CO* system except for sunflower in the 2016/2017 campaign, where the *OR* system produced more (Table 2).

3.5 Discussion

The objective of the present article was to investigate soil fertility as influenced by different agroecosystem management options and tillage operations. Soil fertility is a multifaceted phenomenon, featured by short- and long-term dynamics. To address this complexity, we have measured 13 different indicators monitoring chemical, physical and biological soil properties. These indicators will be discussed in order of their statistical significance and interpretability.

Three indicators hold robust, statistically significant and non-controversial results.

(i) higher available P_2O_5 in the topsoil profile (0–30 cm) was found in conventionally managed soils;

(ii) root density on a 0–100 cm profile was higher in organically managed soils;

(iii) earthworm abundance increases while moving from plowing to chisel plowing and disk harrowing.

Concerning chemical fertility, phosphorus plays a key role in the long-term comparison of conventional and organic farming systems as highlighted by Gosling and Shepherd (2005). In the *OR* soils of our experimental site, P_2O_5 decreased by about 40 % over 25 years (Migliorini et al., 2014) and its current availability is low from an agronomic point of view (Giandon & Bortolami, 2007). This P_2O_5 deficiency is unsurprising as the *OR* fields had not been amended or treated with P-rich materials for 25 years, while high-input agriculture overcomes this problem by constantly adding P with fertilizers. In organic agriculture, soil fertility and productivity rely on biological processes carried out by soil microbiome. Among soil microorganisms, arbuscular mycorrhizal fungi (AMF) may play an important role by compensating for the reduced use of fertilizers, particularly phosphorus. Previous studies carried out at MoLTE (Bedini et al., 2013) showed that AMF population activity was higher in organically managed fields and increased with time since transition from conventional to organic farming. Given that the non-availability of phosphorous is exacerbated in calcareous soils with high levels of mineralization in Mediterranean climates, we believe that further research should focus on AMF bio-functionality in such pedo-climates.

Concerning the biological indicators, higher root densities were observed in the *OR* system for each MAN^*TIL combination (Table 2, row 12). Nevertheless, *OR-plw* soil profile shows less root density in shallow layers but a slower decay of root density along the profile compared to the soil under reduced tillage (*chp, dsh*), thus indicating a greater volume of soil containing plant roots. This is in line with the results of Peigné et al. (2018) who found a greater root density in the first 5 cm soil layers under very superficial and superficial tillage compared to ploughing treatments, and the opposite below 20 cm depth.

Earthworm abundance increased in the order *dsh*>*chp*>*plw* (Table 4) indicating a positive effect of reduced tillage on the earthworm population as stated

by Kuntz et al. (2013). The time regression suggests a higher resiliency of the earthworm population in the *OR* soils as shown in Figure 3.
Moreover, considering the predictions of GLM model, we learn that earthworm abundance is higher in organically managed soils on a 0–30 cm profile. A similar clear positive trend for earthworm abundance in organic agriculture is reported by Bai et al. (2018). The reason why the earthworm abundance increased from November 2015 to March 2017 in *CO* system is not easy to address. Practices performed in *CO* fields, such as tillage, chemical fertilization, chemical hoeing, i.e. events which could affect the presence of the earthworms, were the same in both 2015/2016 and 2016/2017 agricultural campaigns. On the other hand, a possible trend in *OR* system could not be observed since the experimentation had to follow the main crop (barley and sunflower) in the rotation. Thus, the earthworms sampling of 2015/2016 campaign has been done in the *FIELD*s 1 and 3 while the sampling of 2016/2017 campaign has been done in the *FIELD*s 2 and 4. In line with findings of Pelosi et al. (2015), this study highlighted that a long-term approach is required to assess the effects of cropping systems on earthworm abundance and distribution since these types of macro-organisms need time to adapt and respond to different soil conditions. Results for earthworms and root density support the presence of an active biotic community in organic fields at MoLTE, as further witnessed by previous and ongoing MoLTE studies on soil microorganisms (Bedini et al., 2013), plants and above-ground insect predators Moschini et al. (2012), ants' and coleopters' biodiversity (study in progress), soil microbiome biomass and activity (manuscript submitted).

Being the most relevant and interpretable results shown, we now discuss those parameters which were found significantly different but whose interpretability is somewhat more obscure or difficult.

Concerning physical indicators, organic soils showed to be less resistant to penetration (0–80 cm profile), as found by by Bassouny and Chen (2016). The greater volume of soil containing roots in *OR* soils (Figure 4), thus a different distribution of OM along the profile, may account for the better structure (read: ease of penetration). According with Lotter et al. (2003), a greater amount of OM in deeper layers, which is only here hypothesized, could account for higher water retention, thus leading to a softer and better-structured soil. However, soil sampled in *CO* fields has more stable aggregates (Table 13). Since the fragments released by the aggregates on submersion are always significantly greater in *CO* than *OR*, it must be concluded that stronger cements are present in *CO* but it is not easy to ascertain the reason why this might be so. This is in contrast with the findings of various studies which state that organic farming significantly improved aggregate stability as compared to conventional systems (Gerhardt, 1997; Jordahl & Karlen, 1993; Mäeder et al., 2002; Schjønning et al., 2002; Siegrist et al., 1998; Williams & Petticrew, 2009). There is a close relationship between OM content and aggregate stability (Loveland & Webb, 2003). The amount of OM is usually considered to be one of the factors principally responsible for aggregate stability as it forms humo-mineral complexes, but in this case there was no significant difference in OM amounts found between *CO* and *OR* fields (Table 2). Thus, it can be assumed that the difference in aggregate stability is due to the strength of bonds between OM and solid phase which can be attributed, for example, to the quantity of oxides that are considered one of the main binding agents affecting OM stabilization (Six et al., 2004). From another point of view *OR* soil showed higher percentage of micro-aggregates (<20 µm), i.e. a long-term organic carbon reservoir as indicated by many authors (Ŝimanský & Bajĉan, 2014; Six et al., 2004). No clear explanation of how and how much soil management and tillage affect aggregates stability at the MoLTE was found.

Soil profile assessment results confirm that *OR* management lead to a better soil structure in the 15–40 cm layer (Genesio, 2018), which confirms that *OR* systems seems capable of leading to long-lasting soil fertility as suggested by by Mäeder et al. (2002).

Yield was generally higher in *CO* system for both barley and sunflower and this is in line with the findings of many other authors who observed a decrease in yield in *OR* systems as compared to *CO* systems (Gomiero, 2018; Mäeder et al., 2002; Muller et al., 2017; Ponisio et al., 2015). However, the short-term effect due to different tillage intensity was not observed. This is in contrast with the findings of the meta-analysis of Cooper et al. (2016), who found that reducing tillage intensity in organic systems reduced crop yields by an average of 7.6%. The 2016/2017 campaign was characterized by a long period of drought which compromised sunflower productivity and in this scenario the *OR* system produced more than twice that of the *CO* system. In this extreme climatic conditions, barley showed a better drought tolerance since it was harvested at the beginning of July while sunflower remained in the field in July and August which have been the two driest months of 2017. Even if this result suggests a greater resilience of organically managed systems, a long-term yield assessment is needed to support this hypothesis. For example Smolik et al. (1995) and Lotter et al. (2003) found that yield on long-term is less variable in organically managed cropping systems.

Among the 13 explored indicators, porosimetry, bulk density and spade test gave either not significant results or of dubious utility. As it concerns porosimetry, the most obvious reason for not finding significant differences is the low number of samples analyzed which in turn is due to financial limiting factors. Soil bulk density, measured either with *Core* or *Clod* methods, showed some significant results, but the differences were so tiny that gave substantially no usable information. The difference in absolute values for bulk density between *Core* and *Clod* methods is most probably due to the dimensions of the specimens under analysis. Indeed, the cores taken in the field ($\sim 850\,\mathrm{cm}^3$) can contain vary large pores, even cracks several centimeters wide, while the peds/aggregates cannot ($\sim 13\,\mathrm{cm}^3$).

The spade test method applied to MoLTE fields showed that the soil structure conditions are generally good for both *CO* and *OR* systems, since a score greater than 3 (B. C. Ball et al., 2017; Cherubin et al., 2017) — indicating a very poor structure — was assigned only four times. Even if the spade test allowed us to obtain information about the shape and dimension of the soil aggregate and the presence of tillage pan, nevertheless significant differences for the two factors of the present experiment were not found.

3.6 Conclusions

In conventionally managed fields, high crop biomass, possibly linked to higher P_2O_5 availability, might lead to a greater aggregate stability. Organic management positively affects soil biological activity and soil penetration resistance along an 80 cm deep profile; therefore it seems capable of causing long-lasting soil fertility.

Different tillage does not affect soil chemical properties while an effect on physical and biological properties was ascertained. Reduced tillage yields harder soils, though it has a positive effect on soil biological properties. In heavy soils subject to dry summer seasons, chisel plowing appeared to be the most balanced tillage option in terms of biological activity and quality of physical structure.

Among the measured indicators for describing the state of soil fertility, our results suggest that available P_2O_5, aggregate stability, soil penetration resistance, time-related earthworm abundance, root distribution and yields are the most worth acquiring and most informative indicators in the MoLTE experiment.

3.6.0.1 Conflicts of interest

None

3.6.0.2 Author contribution

Conceptualization: Ottorino-Luca Pantani, Lorenzo Ferretti, Margherita Santoni, Gaio Cesare Pacini
Formal analysis: Ottorino-Luca Pantani, Lorenzo Ferretti, Simone Massenzio
Investigation: Ottorino-Luca Pantani, Lorenzo Ferretti, Margherita Santoni, Simone Massenzio, Luigi Paolo D'Acqui
Resources: Ottorino-Luca Pantani, Luigi Paolo D'Acqui
Data Curation: Ottorino-Luca Pantani, Lorenzo Ferretti, Simone Massenzio
Writing - Original Draft: Ottorino-Luca Pantani, Lorenzo Ferretti, Margherita Santoni, Simone Massenzio, Luigi Paolo D'Acqui, Gaio Cesare Pacini
Writing - Review & Editing: Ottorino-Luca Pantani, Lorenzo Ferretti, Margherita Santoni, Simone Massenzio, Luigi Paolo D'Acqui, Gaio Cesare Pacini
Visualization: Ottorino-Luca Pantani, Lorenzo Ferretti, Margherita Santoni
Supervision: Gaio Cesare Pacini
Project administration: Gaio Cesare Pacini
Funding acquisition: Gaio Cesare Pacini

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3.6.1 Supplementary data

Table 5: *Sampling dates and sampling scheme (within each plot) for each indicator.*

Date	Sampling scheme	Indicator
Oct 2015	linear	*Core* bulk density, penetrometry
Nov 2015	triangular	earthworm abundance
Mar 2016	triangular	earthworm abundance
Apr 2016[a]	linear, profiles[c]	chemical parameters,[b] spade test, root distribution
Jul 2016	triangular	barley yield
Sep 2016	triangular	sunflower yield
Oct 2016	linear	*Core* bulk density,[d] *Clod* bulk density, penetrometry, total porosity, aggregate stability
Nov 2016	triangular	earthworm abundance
Mar 2017	triangular	earthworm abundance
May 2017	linear	chemical parameters,[b] spade test
Jul 2017	triangular	barley yield
Sep 2017	triangular	sunflower yield

[a] On barley only, because of drought conditions
[b] A composite sample was obtained by gathering sub-samples from *l*, *m*, *h*, sites
[c] Root distribution [d] Sampled on *m* sites only

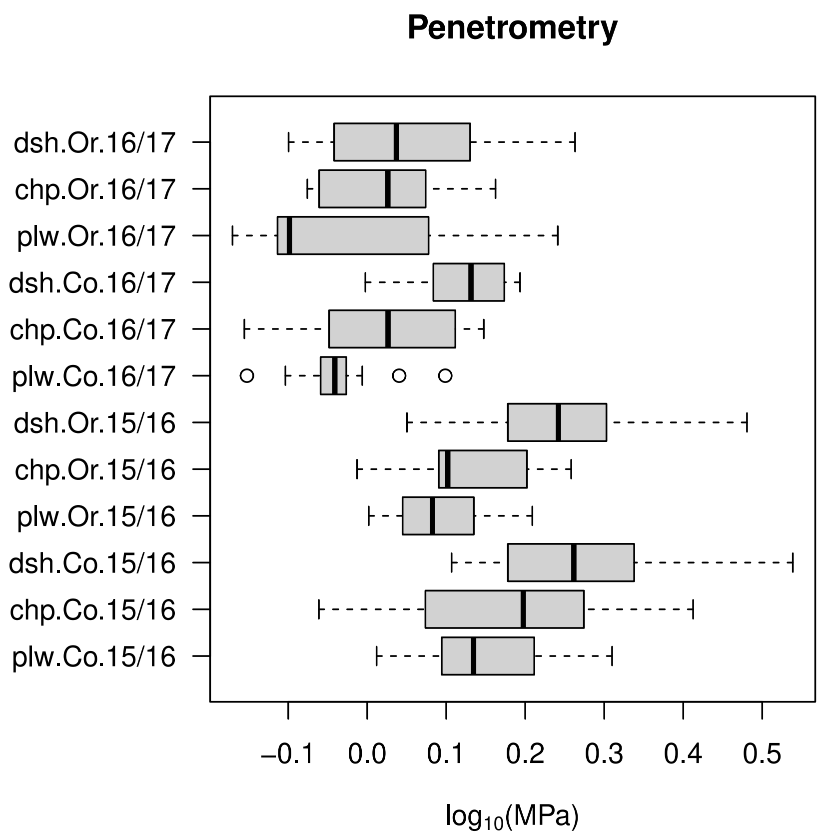


Figure 1: *Mean values of penetrometry data,* $\log_{10}$ *MPa. The mean resistance for CO plowed soils in 2015/2016 was* $10^{0.14}$ *MPa, and decreased to* $10^{-0.01}$ *MPa in 2016/2017 in the same fields. Organic plowed soil were* $10^{0.04}$ *MPa softer than CO plowed ones, while chisel plowed and disk harrowed soils were harder by* $10^{0.05}$ *and* $10^{0.13}$ *MPa, respectively. Formal analysis is reported in Table 11 and Table 3.*

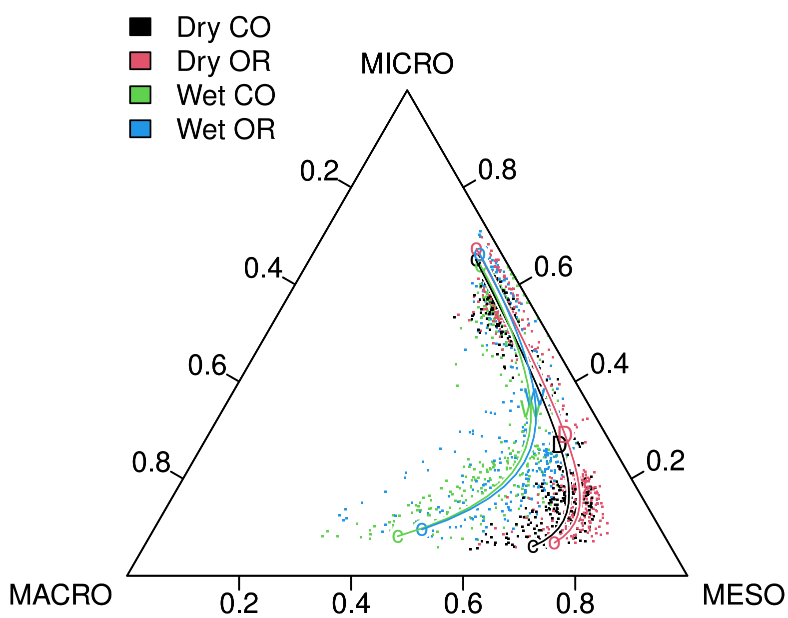


Figure 2: *Evolution of the aggregates' breakdown during the stability test. i) Beginning of the test (points highlighted by* c *and* o*); ii) sonication turned on* (W *and* D*); iii) end of the test* (C *and* O*)* MACRO, MESO *and* MICRO *at triangle vertices indicate diameters greater than 250 µm, within 250 µm and 20 µm and smaller than 20 µm, respectively.* Dry *and* Wet *refers to the humidity of the aggregates and* CO *and* OR *to the type of management. The ternary compositions at i), ii) and iii) are reported in Table 13.*

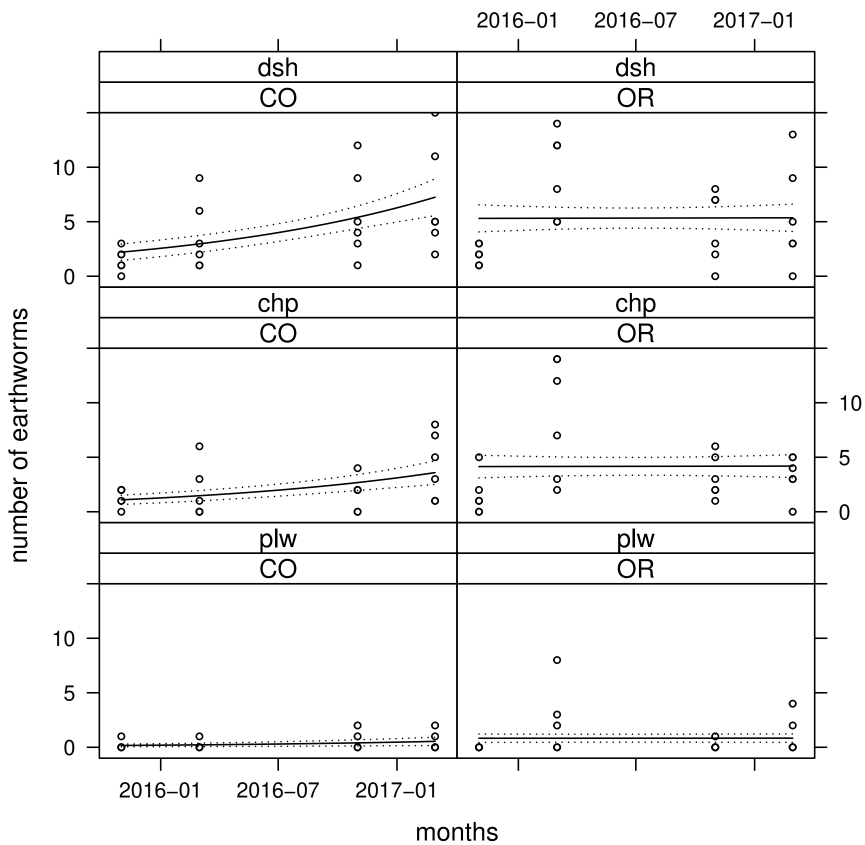


*__Figure 3:__ Graphical representation of the GLM reported in Table 6. Earthworms count as a function of time (from November 2015 to March 2017) as influenced by management (*CO = *Conventional,* OR = *Organic) and tillage (*plw = *plowing,* chp = *chisel plowing,* dsh = *disk harrowing). Points are field experimental data, solid lines represent the expected number of earthworms as estimated by the GLM model, dotted lines are the confidence limits (0.95 conf. level).*

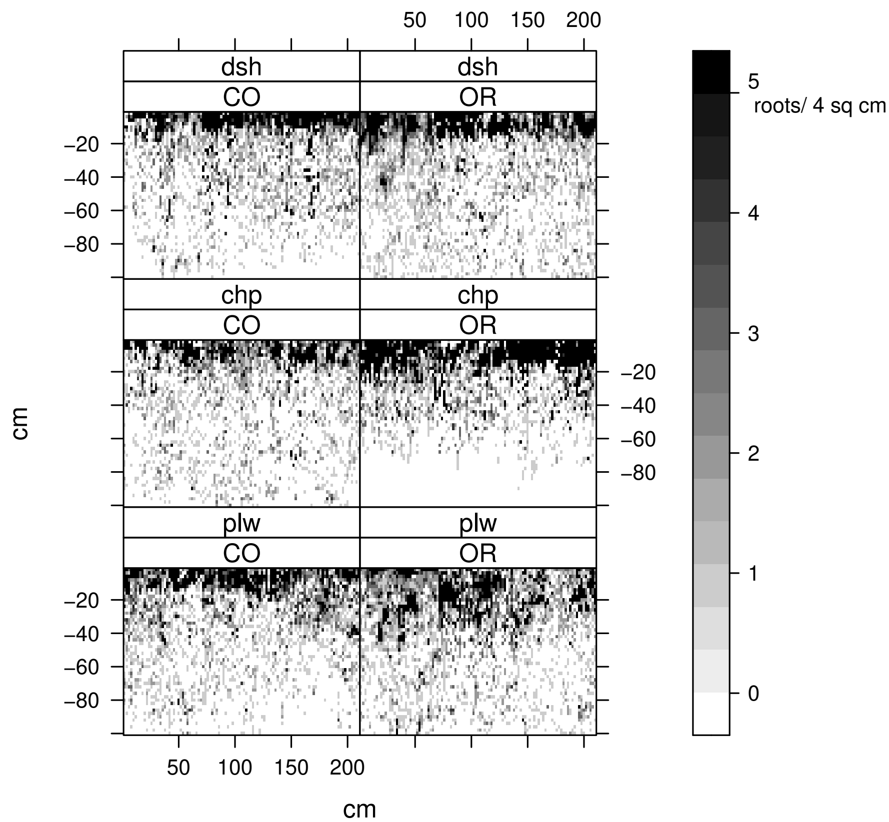


Figure 4: *Root distribution within six soil profiles, as influenced by management (*CO= *Conventional and* OR = *Organic) and tillage (*plw = *plowing,* chp = *chisel plowing,* dsh = *disk harrowing). Each dot represents* $4\,cm^2$ *of the plastic net used for counting the roots.*

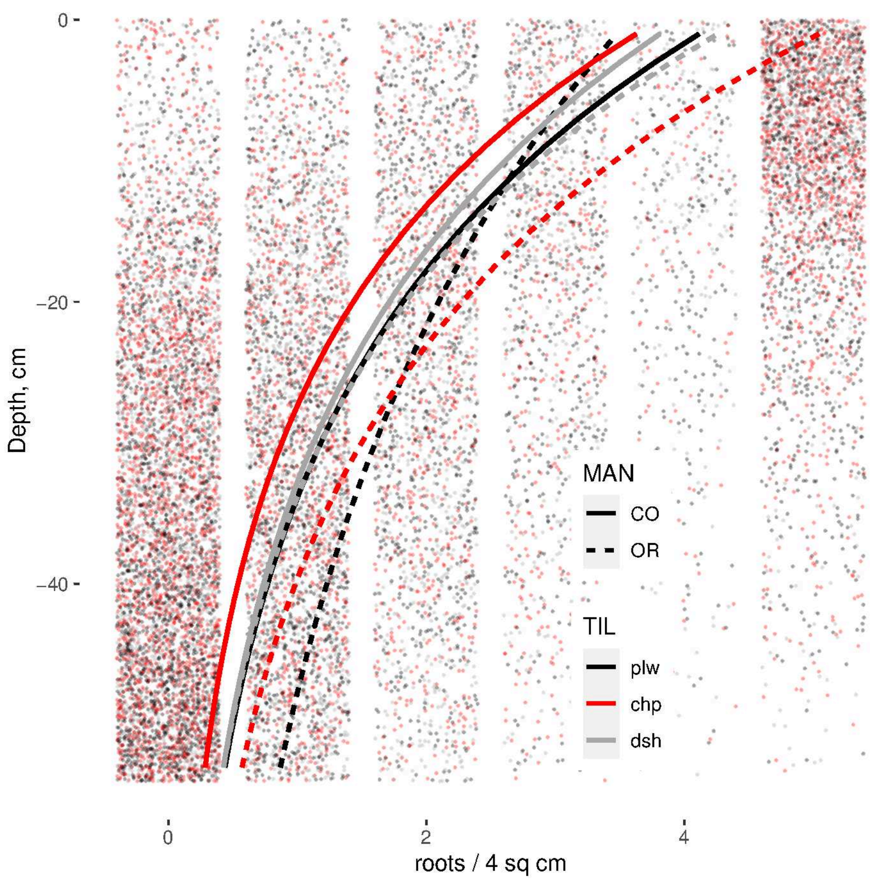


Figure 5: *Root distribution in the first 60 cm of the soil profile as influenced by management* (CO = *Conventional,* OR = *Organic) and tillage* (plw = *plowing,* chp = *chisel plowing,* dsh = *disk harrowing). Curved lines are the expected root number along the depth of the profile, as estimated by the model Equation 3. Each vertical band encompasses a class of the sampled root number (e.g. the rightmost band at x=5 shows the number of cells with 5 roots counted along the depth of soil profile).*

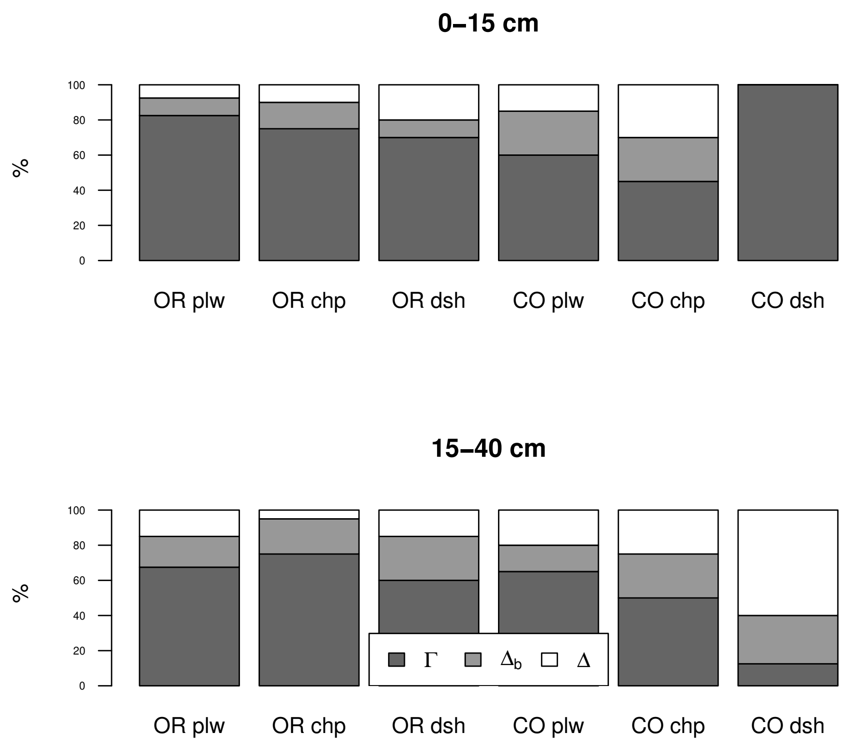


Figure 6: *Percentage of clods with a loose structure (Γ), clods with few biological macropores (Δ_b) and clods with no visible structural porosity (Δ) for each* MAN*TIL *combination along soil depth.*

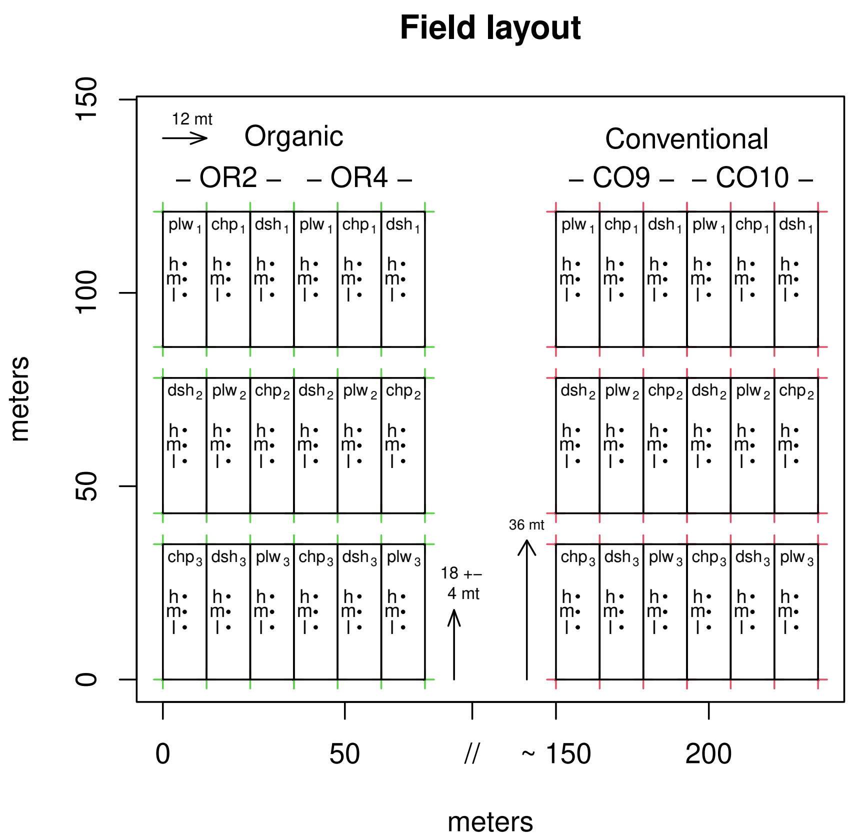


Figure 7: *Sketch of the field layout used from year 2015 to year 2017: the abbreviations* plw, chp *and* dsh *indicate plowing, chisel plowing and disk harrowing while numbers subscripted* $_{1,2,3}$ *indicate the replicate* (REP)*. The abbreviations* OR *and* CO *indicate organic and conventional managed fields, while 2,4,9,10 indicate the number of a single field which is composed by 9 plots. Each plot is 12 mt wide and 36 mt long. In the middle of each plot a sampling site* m *was marked, together with two points 4 mt apart (*h, l*). Further details can be retrieved at https://www.dagri.unifi.it/index.php?module=CMpro&func=viewpage&pageid=475&newlang=eng.*

Table 6: *Concise description of the models used to fit the data.*

Indicator	ANOVA	Summary	Model class	Formula, R notation
P_2O_5, OM, N	Table 2		Linear	$Y \sim$ FACTOR[a]
Spade test, yields	Table 2		Linear	$Y \sim$ FACTOR[a]
BD Clod	Table 8	Table 9	Linear	$Y \sim$ MAN + TIL
BD Core	Table 7		Linear	$Y \sim$ YEAR + MAN + TIL
Penetrometry	Table 11		Linear	$Y \sim$ YEAR * MAN * TIL
Porosity	Table 10		Linear	$Y \sim$ MAN * TIL
Aggregate stability	Table 12	Table 13	Compositional	$Y \sim MAN + MINUTE + I(MINUTE^2)$
n. of earthworms	Table 14	Table 4	General Linear Model	$Y \sim$ MAN + TIL + days + MAN:TIL + MAN:days
Root distribution	Table 16	Table 15	General Linear Model	$Y \sim$ DEPTH.cm * MAN * TIL

[a] In order to perform the Tukey test, indicators listed in Table 2 were analyzed by a FACTOR with 12 levels, as resulting from the experimental factors, *Management*, *Tillage* and *Year*.

Table 7: *ANOVA table for bulk density of the soil as measured with the* Core *method.*

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	1	0.021	0.021	1.817	0.182
Management	1	0.048	0.048	4.100	0.047
Tillage	2	0.002	0.001	0.093	0.911
Total	66	0.775	0.012		

Since the linear model with interactions between experimental factors was not significantly different from the simpler one withouth them (Pr(>F) = 0.74), the linear model considered only the main factors and was in the form

$$y \sim \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \epsilon \quad \text{(a)}$$

Where:
y = bulk density
β_0 = mean
x_1 = *Year*, two levels: *2015, 2016*;
x_2 = *Management*, two levels: *Conventional, Organic*
x_3 = *Tillage*, three levels: *plowing, chisel plowing, disk harrowing*
ϵ = residuals

Table 8: *ANOVA table for bulk densities as measured with* Clod *method.*

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Management	1	0.026	0.026	6.310	0.014
Tillage	2	0.047	0.024	5.829	0.004
Total	104	0.423	0.004		

Similarly to what was found for the *Core* results, no interactions between experimental factors were found (Pr(>F) = 0.8). Since the data were collected in 2016/2017 only, the fitted model used for the analysis was as model Equation a but without the $\beta_2 x_2$ term.

Table 9: *Mean values of bulk densities ($g\,cm^{-3}$), as measured by* Clod *method* Management *and* Tillage.

Management	Tillage	Mean	Std.Dev	n	Tukey
Conventional	plw	1.93	0.06	18	a
	chp	1.90	0.06	18	ab
	dsh	1.89	0.05	18	ab
Organic	plw	1.90	0.07	18	ab
	chp	1.87	0.07	18	ab
	dsh	1.84	0.06	18	b

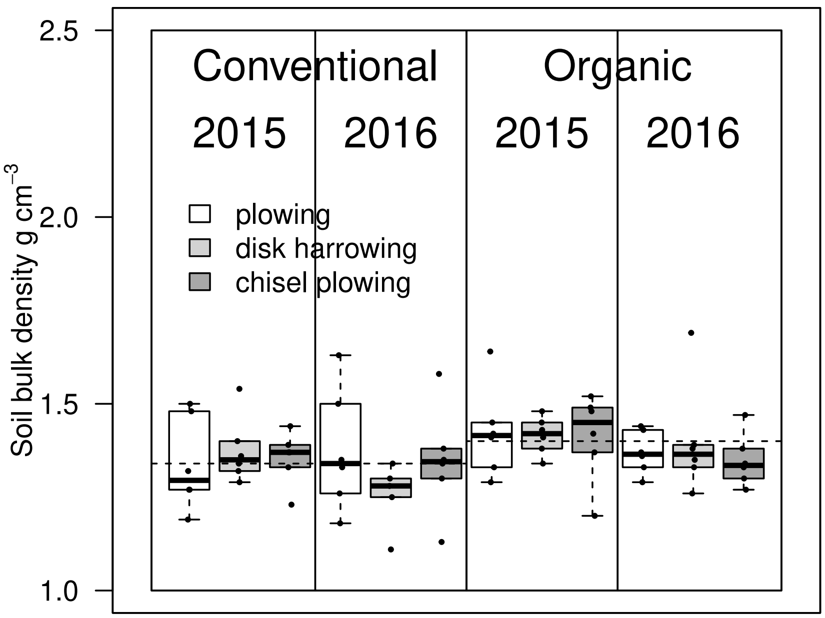


Figure 8: Bulk density measured with Core *method during the 2015/16 campaign, grouped by management (*CO *=* Conventional*,* OR *=* Organic*) and tillage (*plw *=* plowing*,* chp *=* chisel plowing*,* dsh *=* disk harrowing*). Dashed lines are drawn at the means of the two management systems.*

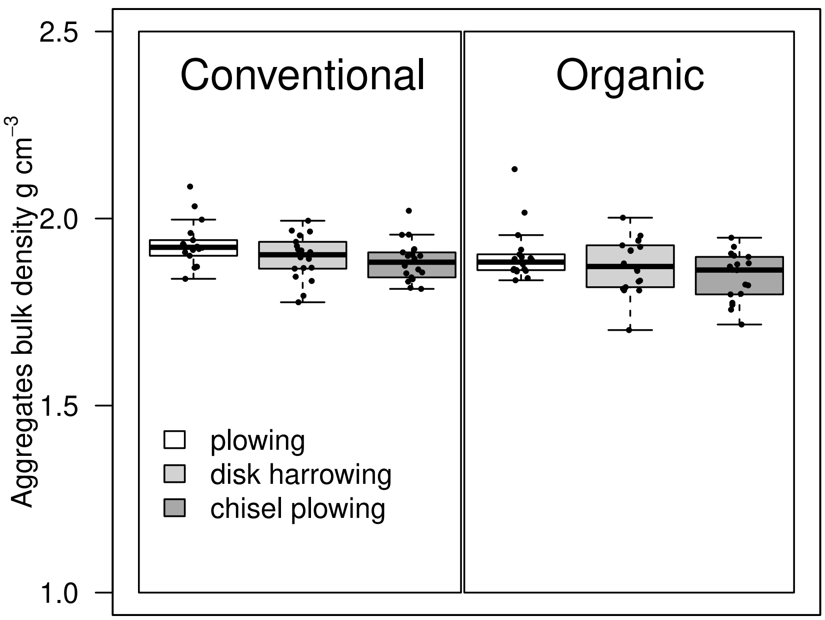


Figure 9: *Bulk density measured with* Clod *method during the 2015/16 campaign, grouped by management (*CO *=* *Conventional,* OR *=* *Organic) and tillage (*plw *=* *plowing,* chp *=* *chisel plowing,* dsh *=* *disk harrowing).*

Table 10: *ANOVA table for total porosity ($mm^3\,g^{-1}$) as measured with Hg porosimetry.*

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Management	1	2.05	2.05	0.00	0.9562
Tillage	2	2448.13	1224.07	1.88	0.1953
Interaction	2	485.96	242.98	0.37	0.6966
Residuals	12	7824.13	652.01		

Table 11: *ANOVA table for penetrometry data*

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	1	1.285	1.285	143.355	$< 10^{-3}$
Management	1	0.072	0.072	8.061	0.005
Tillage	2	0.634	0.317	35.360	$< 10^{-3}$
Total	211	1.891	0.009		

Table 12: *ANOVA table for Particle Size Distribution of soil aggregates as a function of time (minutes) and* management *(CO and OR).* Intercept *is the composition at time zero.*

NAME	Df	Pillai	approx F	num Df	den Df	Pr(>F)
Dry aggregates						$< 10^{-3}$
Intercept	1	0.978	18863.734	2	857	$< 10^{-3}$
Management	1	0.103	49.269	2	857	$< 10^{-3}$
Time	1	0.940	6718.414	2	857	$< 10^{-3}$
Time^2	1	0.192	101.986	2	857	$< 10^{-3}$
Residuals	858					$< 10^{-3}$
Wet aggregates						$< 10^{-3}$
Intercept	1	0.919	4706.239	2	835	$< 10^{-3}$
Management	1	0.057	25.022	2	835	$< 10^{-3}$
Time	1	0.916	4525.724	2	835	$< 10^{-3}$
Time^2	1	0.190	97.881	2	835	$< 10^{-3}$
Residuals	836					$< 10^{-3}$

Table 13: *Expected particle size distribution produced by aggregates during their disgregation.As expected, immediately after submersion, the aggregates show a composition characterized by a larger percentage of coarser dispersed fractions. In fact the compositions at time zero - letters* c *and* o *in Figure 2 - shift towards the MACRO side of the triangle. As time passes, the compositions shift towards the MESO apex along the mean values indicated until they reach the points marked with* D*, when the ultrasonic transducer was turned on, and finally, after 23 minutes, they reach the compositions marked by* C *and* O*, where the suspended MICRO particles are at their maximum of around 65 %.*

Management	Stage	Macro > 250um (%)	Meso (%)	Micro < 20um (%)
Dry aggregates				
Conventional	Start	24.5	69.4	6
	Ultrasonication On	9.3	63.6	27.1
	End	5.2	29.7	65.1
Organic	Start	20.3	72.9	6.8
	Ultrasonication On	7.3	63.6	29.1
	End	4	28.7	67.4
Wet aggregates				
Conventional	Start	47.6	44.2	8.1
	Ultrasonication On	10.8	54.9	34.3
	End	5	31.2	63.9
Organic	Start	42.6	47.9	9.5
	Ultrasonication On	8.8	54.3	36.8
	End	3.9	29.8	66.3

Table 14: *Deviance analysis of the model describing the expected number of earthworms as explained by* Management*,* Tillage *and* Days *from the first sampling date.*

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL			143	593.068	
MAN	1	15.481	142	577.588	$< 10^{-3}$
TIL2	2	188.438	140	389.150	$< 10^{-3}$
true.days	1	13.790	139	375.360	$< 10^{-3}$
MAN:TIL2	2	6.385	137	368.975	0.041
MAN:true.days	1	20.280	136	348.695	$< 10^{-3}$

Table 15: *Summary of the model describing the root density as explained by* Management *and* Tillage *and depth. The expected number of roots per* $4\,\mathrm{cm}^2$ *is given by* $e^{Estimate}$*, as predicted by the model Equation 3.*

	Estimate	Std. Error	z value	Pr(>|z|)
CO-plw	1.458	0.023	62.145	$< 10^{-3}$
Depth, - cm	0.043	0.001	40.657	$< 10^{-3}$
OR-plw	-0.186	0.033	-5.589	$< 10^{-3}$
CO-chp	-0.120	0.035	-3.453	$< 10^{-3}$
CO-dsh	-0.078	0.034	-2.303	0.021
-cm * OR	-0.016	0.001	-11.655	$< 10^{-3}$
-cm * CO-chp	0.006	0.002	3.634	$< 10^{-3}$
-cm * CO-dsh	-0.001	0.002	-0.596	0.551
OR-chp	0.508	0.047	10.816	$< 10^{-3}$
OR-dsh	0.299	0.047	6.343	$< 10^{-3}$
-cm * OR * chp	0.009	0.002	4.486	$< 10^{-3}$
-cm * OR * dsh	0.018	0.002	8.929	$< 10^{-3}$

The output of the GLM model (Table 15) explains how the factors of each variable affect the root distribution.

The general formula of the GLM model used for the analysis is:

$$y \sim \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{1,2} x_{1,2} + \beta_{1,3} x_{1,3} + \beta_{2,3} x_{2,3} + \beta_{1,2,3} x_{1,2,3} + \epsilon \quad (3)$$

Where:
y = number of roots per $4\,\mathrm{cm}^2$;
β_0 = Root' number at 0 cm;
x_1 = *Depth*, cm;
x_2 = *Management*, two levels: *Conventional, Organic*
x_3 = *Tillage*, three levels: *plowing, chisel plowing, disk harrowing*
ϵ = residuals

To avoid working with a very complex model and since root distribution was very slightly affected by profile's width, this variable was not included.

The analysis of deviance table based on the GLM model is complementary

Table 16: *Deviance analysis of the model describing the root density as explained by* Management *and* Tillage. *Depth is in -cm.*

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
			17009.000	37256.901	
Depth	1	10262.725	17008.000	26994.176	$< 10^{-3}$
MAN	1	331.351	17007.000	26662.825	$< 10^{-3}$
TIL	2	36.761	17005.000	26626.063	$< 10^{-3}$
Depth * MAN	1	71.205	17004.000	26554.858	$< 10^{-3}$
Depth * TIL	2	128.240	17002.000	26426.618	$< 10^{-3}$
MAN*TIL	2	192.259	17000.000	26234.359	$< 10^{-3}$
Depth*MAN*TIL	2	80.077	16998.000	26154.283	$< 10^{-3}$

to the output of GLM and shows that the portion of deviance out of the total deviance explained by each of the variables and their interaction is statistically significant ($p < 0.05$). This means that depth (Depth), management (MAN) and tillage (TIL) affect root distribution along the soil profile.

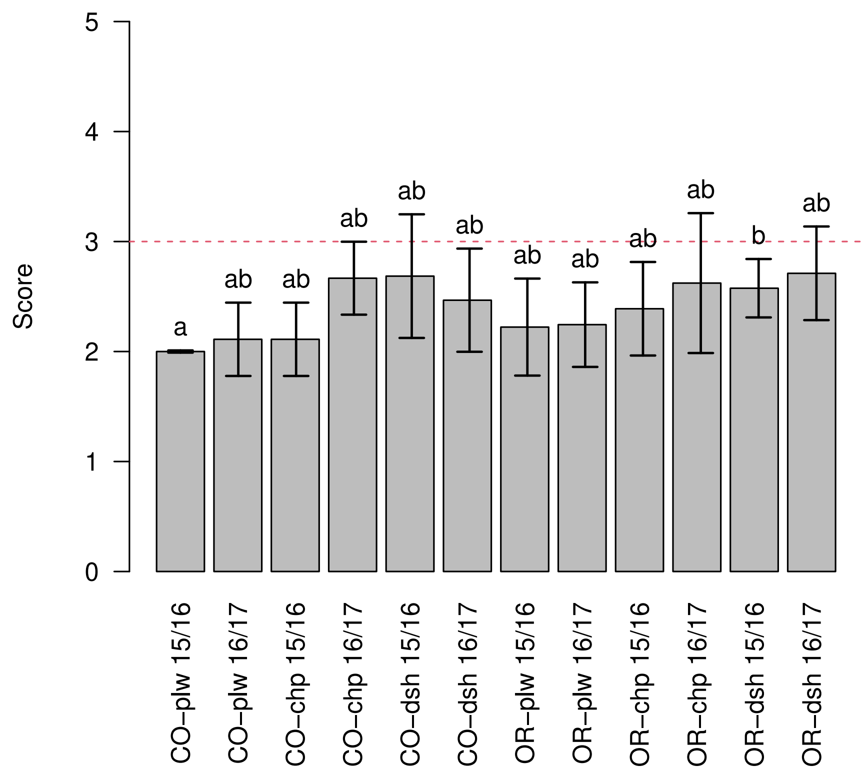
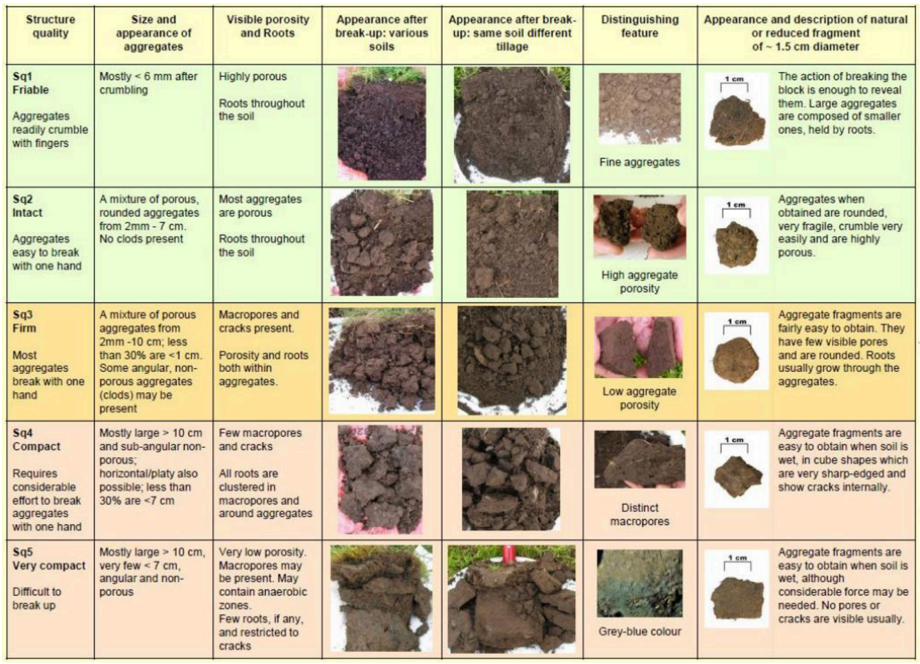


Figure 10: Spade test *score as influenced by management (*CO *=* *Conventional,* OR *= Organic) and tillage (*plw *= plowing,* chp *= chisel plowing,* dsh *= disk harrowing) in 2015/16 and 2016/17 campaigns. The data were not normal: letters indicate the results of a Wilcoxon pairwise comparisons, Bonferroni's method adjusted p-values.*



Structure quality	Size and appearance of aggregates	Visible porosity and Roots	Appearance after break-up: various soils	Appearance after break-up: same soil different tillage	Distinguishing feature	Appearance and description of natural or reduced fragment of ~ 1.5 cm diameter
Sq1 Friable Aggregates readily crumble with fingers	Mostly < 6 mm after crumbling	Highly porous. Roots throughout the soil			Fine aggregates	1 cm. The action of breaking the block is enough to reveal them. Large aggregates are composed of smaller ones, held by roots.
Sq2 Intact Aggregates easy to break with one hand	A mixture of porous, rounded aggregates from 2mm - 7 cm. No clods present	Most aggregates are porous. Roots throughout the soil			High aggregate porosity	1 cm. Aggregates when obtained are rounded, very fragile, crumble very easily and are highly porous.
Sq3 Firm Most aggregates break with one hand	A mixture of porous aggregates from 2mm -10 cm; less than 30% are <1 cm. Some angular, non-porous aggregates (clods) may be present	Macropores and cracks present. Porosity and roots both within aggregates.			Low aggregate porosity	1 cm. Aggregate fragments are fairly easy to obtain. They have few visible pores and are rounded. Roots usually grow through the aggregates.
Sq4 Compact Requires considerable effort to break aggregates with one hand	Mostly large > 10 cm and sub-angular non-porous; horizontal/platy also possible; less than 30% are <7 cm	Few macropores and cracks. All roots are clustered in macropores and around aggregates			Distinct macropores	1 cm. Aggregate fragments are easy to obtain when soil is wet, in cube shapes which are very sharp-edged and show cracks internally.
Sq5 Very compact Difficult to break up	Mostly large > 10 cm, very few < 7 cm, angular and non-porous	Very low porosity. Macropores may be present. May contain anaerobic zones. Few roots, if any, and restricted to cracks			Grey-blue colour	1 cm. Aggregate fragments are easy to obtain when soil is wet, although considerable force may be needed. No pores or cracks are visible usually.

Figure 11: *VESS method standard indicating the soil structure quality and the score to assign to the soil sample.*

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4 Soil microbiome Biomass, Activity, Composition and CO_2 Emissions in a Long-Term Organic and Conventional Farming Systems

Margherita Santoni[1], Leonardo Verdi[1], Shamina Imran Pathan[1], Marco Napoli[1], Anna Dalla Marta[1], Francesca Romana Dani[1], Gaio Cesare Pacini[1], Maria Teresa Ceccherini[1]

[1] Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali (DAGRI) - University of Florence (Italy)

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4.1 Abstract

The implementation of environmentally friendly agricultural policies has increased the need to compare agricultural aspects of conventional (CON) and organic farming (ORG) systems. The objective of the present work was to compare the effects of an organic and conventional long-term experiment on bacterial and fungal biomass and activity, as well as soil CO_2 emission and readily available nitrogen forms in a soil cultivated with Helianthus annuus L. The microbial biomass was more active and abundant in ORG as well as soil CO_2 emission. Despite being less abundant, fungi were more active than bacteria in both ORG and CON experiments. 16S rRNA gene sequencing showed that the ORG treatment had a significantly greater bacterial richness than CON. *Cyanobacteria*, *Actinobacteria* and *Proteobacteria* were the most abundant phyla contributing more than others to the differences between the two systems. Moreover, the soil NH_4^+ and NO_2^- content twas not significantly different between ORG and CON, while NO_3^- was less in ORG. ORG sunflower yield was significantly less compared with CON. While much remains to be discovered about the effects of these agricultural practices on soil chemical properties and microbial diversity, our findings may contribute to this type of investigation.

Keywords: CO_2 emissions, microbial biodiversity, organic and conventional agriculture, qPCR, soil metagenome

4.2 Introduction

Soil quality has been defined as "the capacity of a soil to function within ecosystem and land-use boundaries, to sustain biological productivity, maintain environmental quality and promote plant and animal health" (Doran & Parkin, 1994). Soil microorganisms play a crucial role in maintaining soil quality; they are generally considered the driving force behind litter decomposition processes and play a major role in numerous ecosystem functions, such as organic matter turnover, nitrogen (N) cycling, nutrient mobilization/immobilization, humification, degradation of pollutants and maintenance of the soil structure (Xue et al., 2006). Soil microbiological properties, such as microbial biomass and metabolic activity, are often measured to obtain immediate and accurate information about changes in soil due to land use and agronomic practices. For these reasons, the microbial biomass can be taken as a sensitive indicator of changes in soil fertility (Campos et al., 2014). For a sustainable environment, it is important to improve existing land management systems in order to minimize environmental problems. Conventional agriculture utilizes fertilizers and herbicides to increase crop yields, but also cause a progressive decline in soil organic matter levels, which affect physical, chemical and biological soil properties (Mäder et al., 2002; Pimentel et al., 1995). The use of herbicides can modify the function and structure of soil microbial communities altering the normal ecosystems functionality, which in turn has important implications for soil fertility and quality (Pampulha & Oliveira, 2006). An active soil microflora which provides accessible nutrients for crops is an important priority in all farming systems. A sustainable alternative to conventional agriculture is organic farming, now quite well received on a global scale and covering approximately 72.3 million hectares in 2021 (Willer et al., 2021). As reported by Mäder et al. (2002), soil microbial biomass, dehydrogenase, protease and phosphatase activities were higher in organic systems than in the conventional systems, indicating a higher overall microbial activity. Nevertheless, the number of long-term field trials comparing organic and conventional systems is limited and still there are only a few investigations about the effects of these two systems on soil microbial properties. The hypothesis at issue is that there is an urgent need to better understand how soil use and management affect microbial activity and soil quality. Long-term field trials can help to better investigate soil quality since changes in soil quality may only become apparent over the long term. Therefore, our analyses were carried out at the Montepaldi Long-Term Experiment (MoLTE, San Casciano Val di Pesa), which is the longest experiment on organic farming anywhere in the Mediterranean area. The objective of the present work was to compare the effects of an organic and conventional long-term experiment on the bacterial and fungal biomass and activity, as well as soil CO_2 emission and readily available N forms in the soil. Moreover, we investigated the composition of the bacterial community through 16S rDNA sequencing and we focused on ammonia-oxidizing bacteria (AOB) by qPCR, as the AOB plays a crucial role in the N cycle being very sensitive to environmental stresses (Ceccherini et al., 2007).

4.3 Material and Methods

4.3.1 Site Description and Experimental Design

The trials were located at the experimental farm of the University of Florence (Montepaldi, San Casciano, Val di Pesa, 11°09'08''E, 43°40'16"N) inside the MoLTE (Montepaldi Long-Term Experiment) site. The MoLTE has been active since 1991 and covers a slightly sloping surface of about 15 ha. The experimental area is characterized by a typical Mediterranean and Sub-Apennines climate with average annual precipitations of 770 mm. The summer period is characterized by dry conditions with high temperatures and little precipitations (Bedini et al., 2013). Annual mean temperatures during the experimentation were 14.2°C (Figure 1). Soil, derived from the Pesa river fluvial deposit, is between silty clay loam and clay loam in terms of texture (Table 1). The MoLTE includes different agroecosystem management (MAN) systems; for this experiment, we considered an organic arable system under organic management (EC reg. 2092/91 and following regulations), where certified organic fertilizers, amendments and green manure were used from 1991 (ORG), and a conventional/ high-input arable system where chemical xenobiotics, mineral and synthetic fertilizers have been applied since 1991 (CON), both cultivated with Helianthus annuus L. The agronomic aspects of the experiment are described in Table 2. Two plots (47 × 132 m each) per management option (MAN - ORG and CON) were considered.

Within each plot, a chronological data collection for each indicator (microbial and chemical analysis, and GHGs emissions) was applied, which corresponded to the most important phenological phases of the sunflower (TIME in days, that is, t0-seedling, t7 and t18-intermediate time, t52-raising, t83-intermediate time, t104-flowering and t138-harvest). For the microbial and chemical analyses, three soil samples with random coordinates (REP) were taken for each MAN × TIME combination. For the monitoring of soil carbon dioxide (CO_2) emissions, the closed static chamber technique was adopted (Verdi et al., 2019) for each MAN × TIME combination.

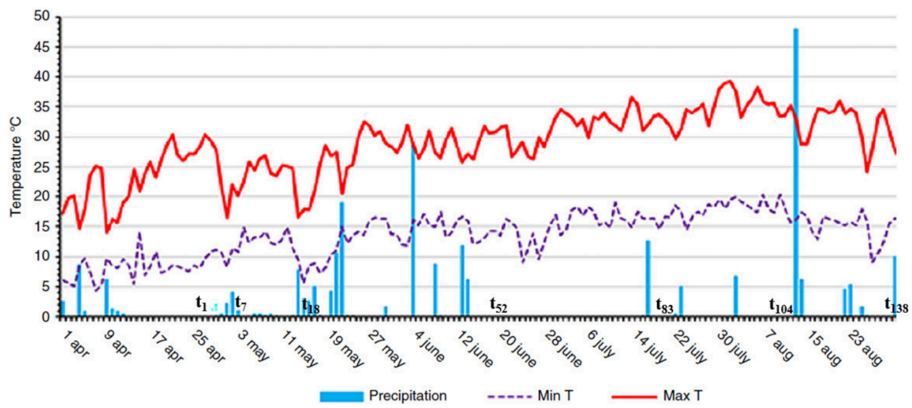


Figure 1: *Maximum and minimum temperature and precipitation trends during the experiment period.*

Table 1: *Soil characteristics of organic (ORG) and conventional (CON) farming systems*

	Units	ORG	CON
Sand	%	20.2	21.0
Silt	%	46.3	44.6
Clay	%	32.9	33.8
Texture		ClayLoam	ClayLoam
pH (H_2O)	8.3	8.3	
Gravel (%)	6.3	6.1	

4.3.2 Soil Sampling for DNA Extraction and Soil Microbial Biomass Determination

Soil samples were collected with a core sampler (3 cm diam.) from the top 15 cm in each plot (ORG and CON). Samples were collected from field in sealed plastic bags and transported on ice to the laboratory. Soil samples were sieved at 2 mm and stored at -20°C until DNA extraction. From each soil sample, 0.5 g of soil

Table 2: *Agronomical details of the MoLTE experiment in 2018. The abbreviations ORG and CON indicate organic and conventional managed plot*

	ORG	**CON**
Previous crop	*Hordeum vulgare, var. Campagne*	*Hordeum vulgare, var. Campagne*
Actual crop	*Helianthus annuus L., var. Toscana*	*Helianthus annuus L., var. LG50.525*
Plant density	55.384 plant/ha	55.384 plant/ha
Date	**ORG**	**CON**
Oct/19/2018	Harrowing	Ploughing
Oct/23/2018	Green manure sowing[a]	-
Oct/24/2018	Subsoiling	-
Apr/20/2018	Green manure incorporation[f]	Harrowing
Apr/26/2018	Sunflower sowing	Sunflower sowing[e] Localized fertilization[b]
Apr/27/2018	-	Chemical weeding[d]
Jun/11/2018	-	Localized fertilization[c]
Jun/12/2018	Weed Hoeing	Weed Hoeing
Sep/05/2018	Harvest	Harvest

[a] Avena sativa L. (40 kg ha^{-1}) and Vicia faba L. var. minor (80 kg ha^{-1}).
[b] 20.10.10 (150 kg ha^{-1}). [c] Urea (150 kg ha^{-1}). [d] DUAL GOLD, p.a S-metolachlor (1.15 lt ha^{-1}). [e] Seeds treated with Apron-xl a.i. metalaxil-m 30.95%.
[f] Harrowing was used for green manure incorporation into the soil.

was used for total DNA extraction by FastDNA Kit for Soil (MPBiomedicals) as described in Ascher et al. (2009). Estimation of soil microbial biomass was carried out on the base of DNA yield, using picodrop-based quantification of double-stranded DNA (dsDNA) and stored at -20°C (Fornasier et al., 2014; Marstorp & Witter, 1999).

4.3.3 Quantitative PCR (qPCR)

Quantitative PCR was performed to determine the 16S rRNA gene copy number of bacteria, the 18S rRNA gene copy number of fungi and the functional gene *amo*A copy number of ammonia oxidizers (AOB) in soil, using 40 ng DNA templates for all the samples. Reactions were performed in an iCycler (Bio-Rad), and the results were analysed with the manufacturer's software (Optical System Software v 3.0a). Amplification was carried out in a 25 µL final volume containing: 2.5 pmol of each primer, 12.5 µL of iQ SYBR Green Supermix (2X) and sterile ddH2O to reach the appropriate volume; three replicates were carried out for each sample. Amplification reactions were performed in 96-well microtitre plates (BioRad); with a known amount of *Bacillus subtilis* BD1512 341f/515r 174 bp PCR fragment previously amplified and purified (Simmons et al., 2007), *Saccharomyces boulardii* (Zambon Italia) FF390/FR1 390 bp PCR fragment (Chemidlin Prévost-Bouré et al., 2011) and *Nitrosolobus multiformis* ATCC 25196 *amo*A1F/2R 490 bp PCR fragment (Ceccherini et al., 2007; Rotthauwe et al., 1997) in each plate were used to develop the standard curve for the respective qPCRs by plotting the logarithm of known concentrations (from 10^{-1} to 10^{-6} ng in 25 µL reaction for eubacteria and fungi; from 10^{-4} to 10^{-9} ng in 25 µL reaction for ammonia oxidizers) against the threshold Cycle (Ct) values. The qPCR program for eubacteria had an initial step of denaturation (3 min, 95°C) followed by 40 cycles of 15 s at 95°C, 30 s at 63°C and 30 s at 72°C; for fungi an initial step of denaturation (3 min, 95°C) followed by 40 cycles of 45 s at 95°C, 30 s at 50°C, 50 s at 70°C, 25 s at 90°C and 4 min at 72°C; for ammonia oxidizers an initial step of denaturation (3 min, 95°C) followed by 40 cycles of 45 s at 95°C, 30 s at 55°C and 50 s at 72°C. After each cycle, a melting curve programmed was run for which measurements were made at 0.5°C temperature increments every 10 sec within a range of 60–100°C.

4.3.4 16S rRNA Gene Sequencing

The V3-V4 region of 16S rRNA gene was amplified using the Illumina bar-coded primer pair 341F/805R (Klindworth et al., 2013) by using a TProfessional thermal cycler (Biometra, biomedizinische Analytik GmbH). The PCR reaction mix (50 µL) contained: 40 ng of template DNA, with KAPA Hifi Hotstart readyMix (Roche). PCR running conditions were as follows: 3 min denaturation at 95°C, followed by 25 sequential cycles each consisting of 30 s at 95°C, 30 s at 55°C, 30 s at 72°C, followed by a final extension step at 72°C for 5 min. PCR products (amplicon size ~550 bp) were purified using a AMPure XP beads (Fisher Scientific) and then quantified by an Invitrogen™ Qubit™ 2.0 Fluorometer (ThermoFisher Scientific). Purified amplicons were used for library preparation and sequencing, according to the Illumina 16S Metagenomic Sequencing Library Preparation guide (downloaded from https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). Paired-end sequencing (2 × 300 bp) was carried out by using a MiSeq System. Since the ORG and CON soils belonged to a long-term experiment (28 years), it can reasonably be considered that the established microflora were stabilized. Moreover, since the purpose of the 16S rDNA sequencing was intended only to highlight any possible differences in the soil bacterial community under the two types of management, DNA replicates (REP) of each sampling time (TIME) were pooled together and considered as a representative sample for each management option (2 MAN × 7 TIME).

4.3.5 Sequencing Data Processing

Paired reads were assembled, quality-filtered and analysed using the pipeline SEED 2.0.3 with the inclusion criteria of mean quality score $\geq$ 32 and length $\geq$ 250 bp. Briefly, chimeric sequences were detected using the de novo VSEARCH algorithm (Větrovsky & Baldrian, 2013) and removed from the dataset. Sequences were then clustered into operational taxonomic units (OTUs) at a 97% sequence identity threshold using the VSEARCH algorithm; consensus sequences were constructed for all clusters (Rognes et al., 2016). Low abundant sequences ($\leq$ 5 of total count) were excluded from further analysis. Identification and the taxonomic assignment were done using representative sequences retrieved from RDP database (Wang et al., 2007) and the NCBI using a 10^{-4} E value threshold. Sequences identified other than bacteria were discarded. The remaining sequences were used to create OTU table and then normalized by dividing sequences of individual OTU. Phylogenetic assignment to bacterial phyla and class level was based on best hits, by dividing the number of sequences belonging to each phylogenetic group by the total number of sequences in the given sample. A Venn diagram was constructed to identify shared and unique OTUs between the two different management practices (ORG vs. CON). Rarefaction and *alpha* diversity of OTUs were performed on re-sampled data sets with the same number of sequences randomly selected from all samples (50.000 sequences) using the SEED 2.0.3 software (Větrovsky & Baldrian, 2013). OTUs with $>$ 0.1% abundance were used to evaluate differences in *beta* diversity.

4.3.6 Soil CO_2 Emissions, Fluxes Estimation and Specific Respiration of Biomass ($mqCO_2$)

For the monitoring of soil carbon dioxide (CO_2) emissions, the closed static chamber technique was adopted. Chambers were constructed as described by Parkin and Venterea (2010) and Verdi et al. (2019). Emissions were monitored using a portable gas analyser (Madur, XCGM 400) as described by Verdi et al. (2019). Gas sampling was carried out inserting a needle, connected to the gas analyser by a polytetrafluoroethylene tube, for one minute. Gas samplings were carried out immediately after chamber closing (t0) and after one hour of gas accumulation (t1) with the chamber closed. Gas samplings were carried out bi-weekly throughout the growing season, from 20th April (cotyledons emergence) until 5th September (physiological maturity) 2018. The ratio of soil CO_2 emissions to the microbial biomass, this latter expressed as DNA yield (Fornasier et al., 2014), has been used similarly to the metabolic quotient (Blagodatskaya et al., 2003) here indicated as $mqCO_2$ and expressed as kg CO_2 per kg DNA yield per hectare of soil. The ratio of soil CO_2 emissions to the bacterial and fungal gene copies (obtained by quantitative PCR) has been considered here as the metabolic activity of these two microbial communities, indicated as Bac qCO_2 and Fun qCO_2.

4.3.7 Soil NH_4^+, NO_2^-, NO_3^- and Readily Mineralizable Organic N Content

Soil samples were analysed to determine the concentration of readily available forms of N in soil (RA-N): ammonium-N (NH_4^+ - N), nitrate-N (NO_3^- - N), nitrite-N (NO_2^- - N) and readily mineralizable organic N (RMO-N). N forms were determined after extraction with the calcium chloride ($CaCl_2$) procedure by Houba et al. (1995), which has the advantage of extraction uniformity for the considered N forms, and it was found being a good extracting solution for organic N readily available for mineralization and plant uptake (Nunan et al., 2001). Thus, 50 g of air-dried soil from each sample was extracted with a 0.01 M $CaCl_2$ solution (Sigma-Aldrich, 97%) at a soil: solution ratio of 1:10. The suspension was shaken for 2 h at 150 rev min^{-1} at room temperature and then filtered through Whatman no. 42 nitrate-free filter paper. The concentrations of N forms in solution were determined by spectrophotometry using a Lambda 20 spectrometer (PerkinElmer). The NO_2^- - N concentration in solution was determined by means of Griess reaction (EPA, 1993). Aliquots of 10 ml of soil extract solution were treated with the Griess reagent (Sigma-Aldrich) containing 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride solution and a 1% sulphanilamide solution in 5% phosphoric acid. The absorbance of the nitrite-containing sample was measured at 540 nm (value A). Aliquots of soil extract solution (25 ml) were treated according to the nitrate copper-cadmium reduction method (APHA, 2000) to reduce nitrate to nitrite. The resulting solutions were treated by means of Griess reaction and then spectrophotometrically analysed at 540 nm as previously described to determine the concentration of NO_2^- - N + NO_3^- - N (value B). Finally, NO_3^- - N concentration (value C) was calculated by subtracting nitrite values (value B - value A). Further aliquots (10 ml) of soil extract solution were treated following the Nessler method (ASTM, 2015) and then absorbance analysed at 420 nm for the determination of NH_4^+ - N concentration (value D). Aliquots (20 ml) of soil extract solution were acid digested for 2 h using 2 ml of H2SO4 (Nunan et al., 2001). The resulting digested solutions were then transferred to 50 ml volumetric flasks, pH adjusted to 7 with 1

N sodium hydroxide and then brought to volume with deionised water. Then, according to (Nollet et al., 2014), the solutions were treated with Nessleration as previously described and analysed at 420 nm for the determination of NH_4^+ - N concentration (value E). RMO-N concentration (value F) was determined by difference between E and D values. Finally, the total RA-N was calculated by summing the N determined after reading A, C, D and F. Quality control (QC) for N measurements includes triplicate analysis of each sample, and 7 of every 50 samples analysed were known QC samples (distilled water blank, 0.5, 2.5, 5, 25, 50 and 250 mg l^{-1}).

4.3.8 Sunflower Yields and Morphological Parameters

Plant morphological parameters were assessed in order to test the effects of different farming systems on sunflower. Crops were harvested on 5th September 2018 in a sampling area of 500 m^2 for the analysis of plant height, flowers diameter, average number of seeds per plant, average weight of seeds per plant and yields (kg ha^{-1}). Three sampling sites with random coordinates were identified in the sampling area. On each coordinate, a two-metre-long ruler was used to collect sunflowers plants. Crop samples were collected in field and dried in a laboratory stove at 80°C for 48 h until constant weight detection for dry weight determination. Dry matter yield was then calculated by averaging the three replicate samples and by standardizing sunflower seeds to tons ha^{-1}.

4.3.9 Statistical Analyses

The analytical process was as follows. The microbial qPCR, chemical and CO_2 results were analysed by linear mixed-effects (LME) models built by lme() function. Analysis of residuals did not show substantial deviation from normality. To compare the models, we used Akaike Information Criterion (AIC) (Sakamoto et al., 1986), choosing the model with the lowest AIC (Pinheiro & Bates, 2000). These analyses were performed using the R statistical software (R Core Team, 2020). The bacterial sequencing data were analysed by PAST 3.03 (Hammer et al., 2001) and R statistical software (R Core Team, 2020). *Alpha* diversity of OTUs was performed by One-way ANOVA followed by Tukey's post hoc test at $p < 0.05$ level of significance to analyse the individual significance. A principal coordinate analysis (PCoA) and PERMANOVA test were conducted based on Bray–Curtis similarity distance to determine the distribution of diversity and statistical significance of beta diversity, respectively. A SIMPER test to estimate which OTUs are responsible more than others for the differences between the two managements was performed.

4.4 Results

4.4.1 DNA Extraction, Soil Microbial Biomass and qPCR

DNA yield, taken as a measure of microbial biomass, showed a similar trend among the two different managements during the time of plant growth (Figure 2). In particular, the amount of DNA was maximum at t18 days and minimum at t104 days for both organic and conventional treatments. However, considering the complete growing season of sunflower, indicated here as t0-138 days, the overall DNA yield was significantly higher in ORG (2.9E + 04 $\pm$ 4.7E + 03 kg DNA ha^{-1} soil) than in CON (2.3E + 04 $\pm$ 7.0E + 03 kg DNA ha^{-1} soil); this latter representing almost 78% of the organic one. The microbial biomass, evaluated as DNA yield, followed the same trend in the two farming systems. By using qPCR, the 16S rRNA (bacteria), 18S rRNA (fungi) and the functional gene *amo*A (ammonia-oxidizing bacteria) copy numbers were evaluated in both the ORG and CON. Looking at the whole period t0-138, bacterial gene sequences were significantly greater in ORG ($1.4 \times 10^{19} \pm 3.7 \times 10^{18}$ copies ha^{-1}) than in CON ($9x10^{18} \pm 4.1 \times 10^{18}$ copies ha^{-1}) samples and the same was for the fungal sequences ($5.7 \times 10^{17} \pm 1.7 \times 10^{17}$ and $3.1 \times 10^{17} \pm 1.9 \times 10^{17}$ copies ha^{-1}, respectively). In general, bacterial gene copies were more abundant than fungal for the two treatments, the fungi representing the 4.2% and the 3.4% of bacteria in ORG and CON samples, respectively. The *amo*A gene sequences (ammonia-oxidizing bacteria) were the smallest number at t7 and the greatest between t83 and t104 in the CON system; the greatest at t18, the least at t0 in the ORG plot (Table 3). Moreover, considering the data for the whole growing season, ammonia oxidizers showed an opposite behaviour to bacteria and fungi; in fact, *amo*A gene copies were significantly less abundant in the ORG ($1.8 \times 10^{16} \pm 5.3 \times 10^{15}$ copies ha^{-1} corresponding to the 38% of the conventional soil) than in the CON farming system ($4.8 \times 10^{16} \pm 2.2 \times 10^{16}$ copies ha^{-1}) and AOB sequences were the 0.1% and 0.5% of the eubacteria in ORG and CON, respectively.

Table 3: *16S rRNA (bacteria), 18S rRNA (fungi) and amoA (ammonia-oxidizing bacteria) sequences per hectare*

Time	**ORG 16S seq $ha^{-1} \pm SD$**	**CON 16S seq $ha^{-1} \pm SD$**	**ORG amoA seq $ha^{-1} \pm SD$**	**CON amoA seq $ha^{-1} \pm SD$**
t0	$1.1 \times 10^{19} \pm 1.3 \times 10^{18}$	$6.8 \times 10^{18} \pm 5.2 \times 10^{17}$	$9.6 \times 10^{15} \pm 1.1 \times 10^{15}$	$3.6 \times 10^{16} \pm 3.0 \times 10^{15}$
t7	$1.3 \times 10^{19} \pm 1.3 \times 10^{18}$	$7.5 \times 10^{18} \pm 1.1 \times 10^{18}$	$1.5 \times 10^{16} \pm 1.7 \times 10^{15}$	$2.9 \times 10^{16} \pm 3.5 \times 10^{15}$
t18	$1.8 \times 10^{19} \pm 7.0 \times 10^{18}$	$1.7 \times 10^{19} \pm 4.5 \times 10^{18}$	$2.4 \times 10^{16} \pm 5.0 \times 10^{15}$	$3.1 \times 10^{16} \pm 5.6 \times 10^{15}$
t52	$1.2 \times 10^{19} \pm 6.5 \times 10^{17}$	$5.8 \times 10^{18} \pm 1.0 \times 10^{18}$	$1.8 \times 10^{16} \pm 1.3 \times 10^{15}$	$4.1 \times 10^{16} \pm 5.9 \times 10^{15}$
t83	$1.6 \times 10^{19} \pm 3.5 \times 10^{18}$	$7.6 \times 10^{18} \pm 1.9 \times 10^{18}$	$2.3 \times 10^{16} \pm 5.2 \times 10^{15}$	$8.2 \times 10^{16} \pm 7.7 \times 10^{15}$
t104	$1.2 \times 10^{19} \pm 2.9 \times 10^{18}$	$6.4 \times 10^{18} \pm 6.8 \times 10^{17}$	$1.9 \times 10^{16} \pm 1.5 \times 10^{15}$	$8.1 \times 10^{16} \pm 6.0 \times 10^{15}$
t138	$1.4 \times 10^{19} \pm 3.4 \times 10^{18}$	$1.2 \times 10^{19} \pm 9.7 \times 10^{17}$	$1.9 \times 10^{16} \pm 2.9 \times 10^{15}$	$3.7 \times 10^{16} \pm 2.7 \times 10^{15}$
Time	**ORG 18S seq $ha^{-1} \pm SD$**	**CON 18S seq $ha^{-1} \pm SD$**		
t0	$3.0 \times 10^{17} \pm 3.6 \times 10^{16}$	$2.0 \times 10^{17} \pm 7.9 \times 10^{15}$		
t7	$5.8 \times 10^{17} \pm 7.2 \times 10^{16}$	$1.7 \times 10^{17} \pm 1.9 \times 10^{16}$		
t18	$6.8 \times 10^{17} \pm 9.0 \times 10^{16}$	$6.9 \times 10^{17} \pm 7.8 \times 10^{16}$		
t52	$5.3 \times 10^{17} \pm 2.9 \times 10^{16}$	$1.5 \times 10^{17} \pm 7.5 \times 10^{15}$		
t83	$6.0 \times 10^{17} \pm 8.7 \times 10^{16}$	$2.2 \times 10^{17} \pm 2.5 \times 10^{16}$		
t104	$8.6 \times 10^{17} \pm 6.2 \times 10^{16}$	$2.8 \times 10^{17} \pm 2.7 \times 10^{16}$		
t138	$4.6 \times 10^{17} \pm 2.6 \times 10^{16}$	$4.7 \times 10^{17} \pm 2.9 \times 10^{16}$		

4.4.2 Soil Carbon Emissions and mqCO_2

CO_2 from aerobic and anaerobic processes, respiration of soil fauna, dark respiration of plants as well as CO_2 from root respiration, are included to the CO_2 fluxes measured with the static chambers and could be considered as community CO_2 production. Despite the similar emissions trend from the two farming systems, ORG showed significantly greater CO_2 emissions than CON (Table 4). Data from the whole growing season (t0-138) showed that the CO_2 evolution was similar between the two farming systems, but it was significantly less in CON (462.97 ± 102.6 kgCO_2-C ha^{-1}) than in ORG (1932.68 ± 216.9 kgCO_2-C ha^{-1}). The ratio of soil CO_2 emission to the DNA yield (mqCO_2) was not constant but varied with time: it was minimal at the beginning and at the end of the growing season and peaked at t83 days for both farming systems. The time interval t52 to t104 showed the greatest activity of the microbial biomass, a sort of *hot moment* more evident in ORG. Considering the data as a mean of the entire sunflower growing season, the microbial activity was significantly less in CON ($3.1 \times 10^{-3} \pm 1.5 \times 10^{-3}$) than in ORG ($9.4 \times 10^{-3} \pm 4.7 \times 10^{-3}$) calculated as kg-C per kg of DNA per soil hectare. We applied the ratio of soil CO_2 emission to the amount of bacterial and fungal gene copies, Bac qCO_2 and Fun qCO_2, respectively, to distinguish the physiological activity of these two microbial communities, considering the whole growing season of the crop. Again, both the bacterial and fungal activities were significantly less in CON ($4.5 \times 10^{-19} \pm 3.5 \times 10^{-19}$ Bac qCO_2 and $1.5 \times 10^{-17} \pm 1.4 \times 10^{-17}$ Fun qCO_2) than ORG ($9.5 \times 10^{-19} \pm 4.0 \times 10^{-19}$ Bac qCO_2 and $2.4 \times 10^{-17} \pm 1.0 \times 10^{-17}$ Fun qCO_2). Thus, the bacterial respiration activity in CON samples was 46.7% of the ORG one, while the fungal respiration activity in CON was 64.9% of the ORG one. Anyway, the fungal respiration activity was significantly higher than the bacterial one.

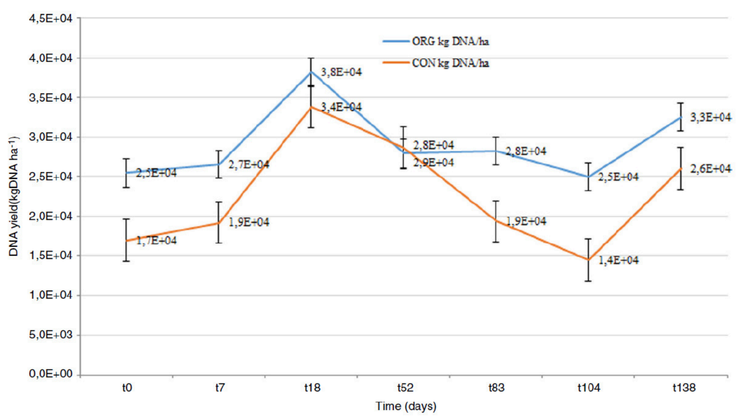


Figure 2: *DNA yield (kg DNA ha^{-1}) per time of sampling*

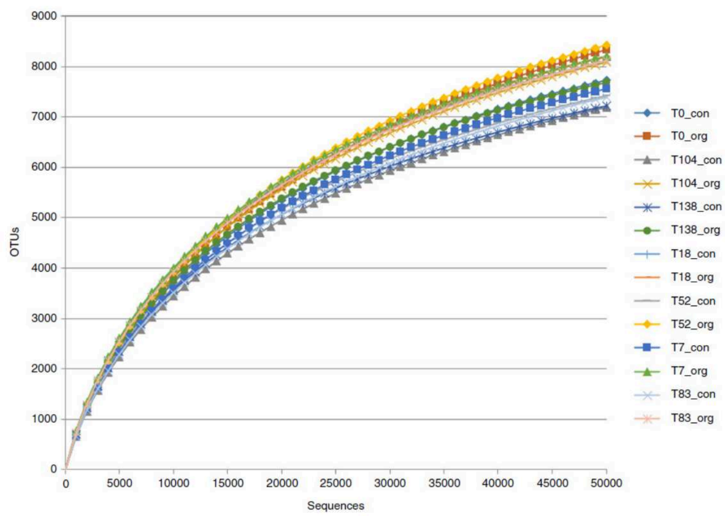


Figure 3: *Rarefaction curves of soil bacterial communities based on observed OTUs at 3% distance for each MAN*TIME combination*

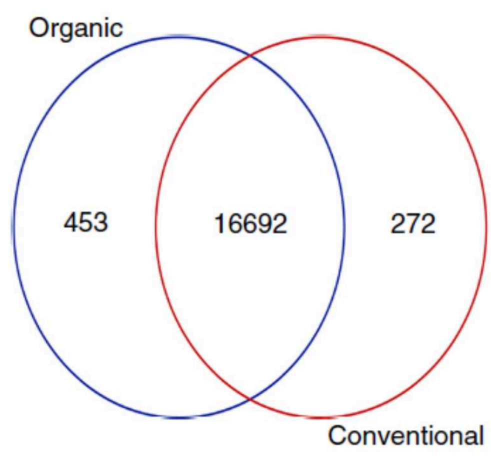


Figure 4: *Venn diagram of exclusive and shared bacterial operational taxonomic units (OTUs) (at the 3% of evolutionary distance) under organic (ORG) and conventional (CON) management*

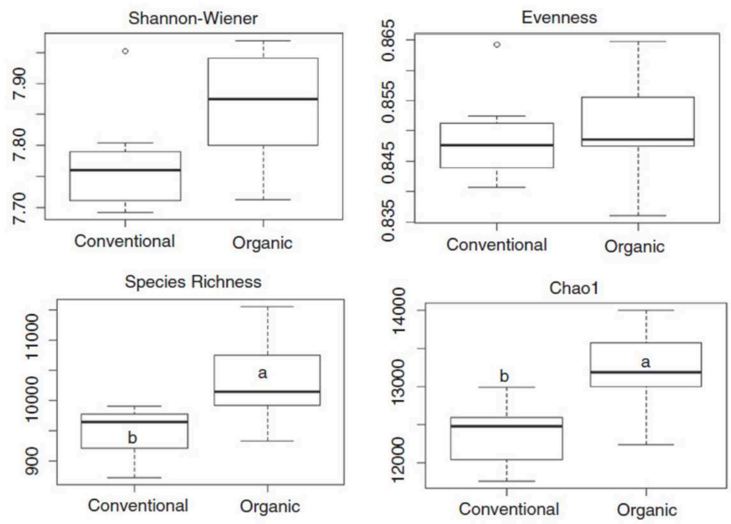
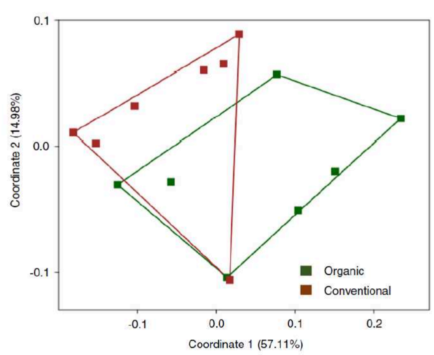


__Figure 5:__ Effect of management practices on variability of extitalpha diversity. Data represent means and errors of three replicates. Significant differences are indicated by different superscript letters (one-way ANOVA followed by Tukey post hoc test, $p < 0.05$).



PERMANOVA	
Permutation N	9999
Total sum of squares	0.3176
Within-group sum of squares	0.2592
F	2.078
p	0.061
p value	
	Organic
Conventional	0.061

__Figure 6:__ Principal coordinate analysis (PCoA) based on Bray-Curtis similarity distance of OTUs with abundance >0.1% of soil bacterial community under organic (ORG) and conventional (CON) management practices. Significant differences detected by permutational ANOVA (PERMANOVA)

Table 4: *Daily CO_2 emission rate at each sampling time for organic (ORG) and conventional (CON) farming system*

Time	ORG kg CO_2-C ha^{-1}	CON kg CO_2-C ha^{-1}
t0	8.09 ± 4.6	2.86 ± 2.2
t7	4.34 ± 2.0	1.57 ± 1.3
t18	12.65 ± 7.2	3.10 ± 1.4
t52	24.32 ± 6.1	4.64 ± 5.4
t83	15.67 ± 7.12	0.82 ± 0.7
t104	14.15 ± 8.8	5.13 ± 2.5
t138	6.71 ± 2.6	0.52 ± 0.9

4.4.3 Bacterial Sequencing Data (*Alpha* Diversity)

After quality filtering, chimera cleaning and removal of low abundant sequences ($\leq$ 5 total count), 2,581,403 16S rRNA sequences and 17,416 OTUs were obtained from a total of 14 samples. The rarefaction curve was reached to saturation for all samples, indicating the sequencing depth was sufficient to cover detectable species in all samples (Figure 3). The Venn diagram revealed that 16,692 OTUs (95.84%) were shared by soil of ORG and CON management practices, while 272 and 453 were exclusive of CON and ORG samples, respectively (Figure 4). Estimated diversity indices, Shannon index, evenness, species richness and Chao1 richness are shown in Figure 5. No significant differences were observed in Shannon index and evenness, while species and Chao1 richness were significantly greater under ORG management compared with the CON one.

4.4.4 Changes in Bacterial Community Structure (*Beta* Diversity)

Beta diversity evaluates how different the population structure is in various environments. PCoA of Bray–Curtis distance was used to analyse the variation in the bacterial community as affected by management practices (Figure 6). The significance level of variation was checked by PERMANOVA. The first two principal coordinators explain a high percentage of variance (~72%, coordinate 1: 57.11% and coordinate 2: 14.98%) with distinction in community structure associated with management practices. Plots revealed that communities were not completely clustered differently under both management practices. PERMANOVA results also showed that there were not significant differences in community structure of bacteria ($F = 2.078$, $p = 0.061$).

4.4.5 Changes in bacterial taxonomic composition

To analyse the effect of management practices on soil bacterial composition, we assessed the bacterial relative abundance at two different taxonomic levels, phylum and family; we showed those present $>$1% (Figure 7a,b). Overall, the *Proteobacteria* phylum (~21%) with classes *alpha*, *beta*, *gamma* and

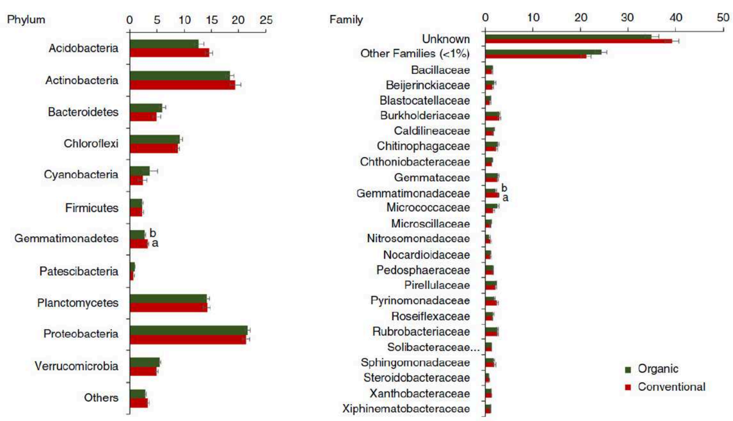


Figure 7: *Variation in bacterial community composition in soil at phylum and family level under organic (ORG) and conventional (CON) management. Data represent means and errors of three replicates. Significant differences are indicated by different superscript letters (one-way ANOVA followed by Tukey post hoc test, $p < 0.05$).*

delta was the most abundant followed by *Actinobacteria* (~19%), *Acidobacteria* (~12%), *Planctomycetes*, *Bacteriodetes*, *Chloroflexi*, *Verrucomicrobia* and *Firmicutes*. No significant differences were observed in the relative abundance of bacteria between the two systems except for *Gemmatimonadetes* (*Gemmatimonadaceae*). A SIMPER test, to estimate which OTUs are responsible more than others for the differences between the two managements, was performed (Table 5). Results showed 22.02% of dissimilarity between ORG and CON management practices. OTU 3, classified as *Tychonema* CCAP 1459-11B belonging to the phylum *Cyanobacteria*, contributed the most to the differences in the bacterial communities (10.65% of total dissimilarity). Other contributing OTUs were OTU 1 *Pseudarthrobacter* belonging to *Actinobacteria*, OTU 6 *Microvirga* belonging to *Proteobacteria*, OTU 4 belonging to *Planctomycetes*, OTU 63 *Microcoleus* PCC-7113 belonging to *Cyanobacteria* and OTU 10 belonging to *Proteobacteria*.

4.4.6 Soil NH_4^+, NO_2^-, NO_3^- and Readily Mineralizable Oganic N Content

The concentration of NH_4^+ - N in soil varied among sampling dates. Over the growing period, the soil NH_4^+ - N concentration ranged from 0.49 to 1.63 mg N kg^{-1} in CON and from 0.28 to 1.35 mg N kg^{-1} in ORG (Figure 8). The soil NH_4^+ - N concentration in CON being significantly greater than that measured in ORG at t0, t7, t52 and t138, while being significantly less at t18, t83 and t104. The average NH_4^+ - N concentration in soil over all sampling dates was greater in CON (1.43 ± 0.56 mg N kg^{-1}) than in ORG treatment (1.35 ± 0.78 mg N kg^{-1}); however, this difference was not significant. The average NH_4^+ - N concentration represented 10.1% and 11.6% of the total readily available N

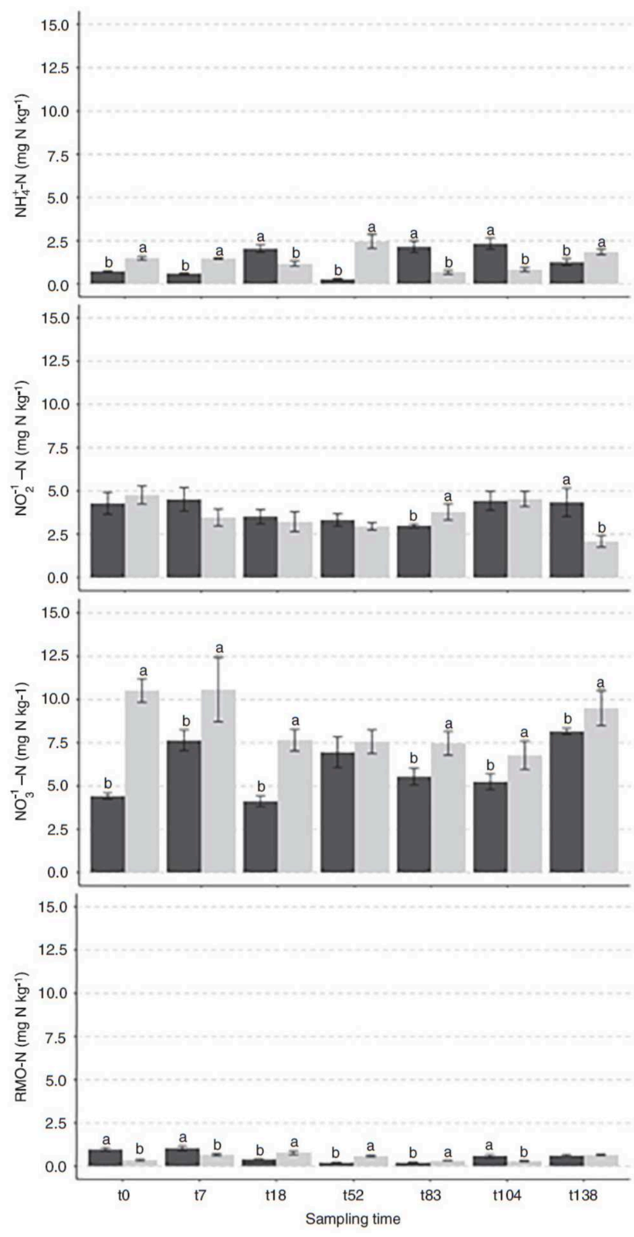


Figure 8: *Variation in soil ammonium-nitrogen (NH_4^- - N), nitrite-nitrogen (NO_2^- - N), nitrate-nitrogen (NO_3^- - N) and readily mineralizable organic nitrogen (RMO-N) in organic (black bars) and conventional (grey bars) treatments at different sampling times (t0-t138)*

Table 5: *Principal OTUs contribute most to change 16S rRNA gene pools under organic (ORG) and conventional (CON) management practices. OTUs with >1% dissimilarity contribution shown in the table*

	Overall dissimilarity: 22.02%						Mean abundance (%)	
OTUs	Av. dissim	Contrib. %	Accession	Phylum	Family	Taxon	ORG	CON
CL000003	2.349	10.65	KX508362	Cyanobacteria	Phormidiaceae	Tychonema CCAP 1459-11B	0.0154	0.00476
CL000001	1.313	5.952	DQ125870	Actinobacteria	Micrococcaceae	Pseudarthrobacter	0.0166	0.011
CL000006	0.5399	2.448	JF417789	Proteobacteria	Beijerinckiaceae	Microvirga	0.00653	0.00486
CL000004	0.5218	2.366	FJ479536	Planctomycetes	WD2101 soil group	uncultured bacterium	0.00743	0.00983
CL000063	0.4899	2.221	KC463683	Cyanobacteria	Coleofasciculaceae	Microcoleus PCC-7113	0.00296	0.0000665
CL000010	0.4684	2.124	FJ478818	Proteobacteria	Burkholderiaceae	Unknown	0.00391	0.00542
CL000038	0.4549	2.063	JQ712903	Cyanobacteria	Phormidiaceae	Unknown	0.00135	0.00264
CL000002	0.3981	1.805	FJ479500	Actinobacteria	Rubrobacteriaceae	Rubrobacter	0.00999	0.0106
CL000011	0.3864	1.752	EU135114	Planctomycetes	WD2101 soil group	uncultured bacterium	0.00345	0.0055
CL000069	0.3693	1.674	FJ444635	Cyanobacteria	Unknown Family	Leptolyngbya EcFYyyy-00	0.000474	0.00228
CL000019	0.3278	1.486	DQ125870	Actinobacteria	Micrococcaceae	Pseudarthrobacter	0.00428	0.00294
CL000021	0.3263	1.479	KC921182	Planctomycetes	WD2101 soil group	planctomycete WWH14	0.00256	0.00405
CL000031	0.3196	1.449	KX508692	Cyanobacteria	uncultured bacterium	Unknown	0.00195	0.00235
CL000007	0.3153	1.429	EU440691	Proteobacteria	Azospirillaceae	Skermanella	0.0056	0.00529
CL000017	0.2916	1.322	EF688375	Actinobacteria	uncultured	uncultured soil bacterium	0.00276	0.00432
CL000009	0.2695	1.222	GQ249621	Actinobacteria	Rubrobacteriaceae	Rubrobacter	0.00511	0.00487
CL000016	0.2666	1.209	JF045039	Actinobacteria	Streptomycetaceae	Streptomyces	0.00337	0.0039
CL000028	0.2388	1.083	CP011509	Proteobacteria	Archangiaceae	Archangium	0.00269	0.00199
CL000094	0.2328	1.056	JQ979031	Cyanobacteria	Unknown Family	Leptolyngbya EcFYyyy-00	0.00112	0.00121

forms in CON and ORG, respectively. The average concentration of NO_2^- - N was 3.54 ± 0.85 mg N kg^{-1} in CON, ranging from 2.09 to 4.76 mg N kg^{-1}, while it was 3.92 ± 0.57 mg N kg^{-1} in ORG, ranging from 3.00 to 4.51 mg N kg^{-1}. The average NO_2^- - N concentration represented 25.5% and 33.3% of the total readily available N forms in CON and ORG, respectively. No significant differences were observed between CON and ORG managements. The concentration of NO_3^- - N in soil ranged from 6.78 to 10.57 mg N kgˆ-1 in CON and from 4.12 to 8.16 mg N kg^{-1} in ORG. When considering the whole period, there was a decreasing trend in the NO_3^- - N concentration in CON, while an increasing trend in ORG was observed. For most sampling dates (t0, t18, t83), the soil NO_3^- - N concentration in CON was significantly greater than ORG. For all sampling dates, it was observed that the concentration of NO_3^- - N in ORG increased as the NH_4^+ - N decreased and vice versa. On the contrary, this relationship was not found in CON. Considering the entire growing season period, the average NO_3^- - N concentration in soil was significantly greater in CON (8.58 ± 1.46 mg N kg^{-1}) than in ORG (6.02 ± 1.47 mg N kg^{-1}). The average NO_3^- - N concentration in soil represented 60.7% and 50.3% of the total readily available N forms in CON and ORG, respectively. During the growing period, the average concentration of RMO-N was 0.53 ± 0.18 mg N kg^{-1} in CON, ranging from 0.3 to 0.78 mg N kg^{-1}, while it was 0.59 ± 0.31 mg N kg^{-1} in ORG, ranging from 0.22 to 1.04 mg N kg^{-1}. The average RMO-N concentration in soil represented 3.8% and 4.9% of the total readily available N forms in CON and ORG, respectively. Throughout the season, the average RMO-N concentration in ORG was greater than in CON. The soil RMO-N concentration in CON resulted being significantly more than that measured in ORG at t18, t52 and t83, while being significantly less at t0, t7 and t104.

4.4.7 Sunflower Yields and Morphological Parameters

Sunflower yields were significantly greater in CON than in ORG (Table 6). However, in both treatments, yields were less than average for sunflower in Tuscany. According to yields, CON had better performances for all measured morphological parameters, except plant height. In particular, flower diameter, average number of seeds per plant and average weight of seeds per plant were 56.8%, 56.9% and 54.4% greater in CON than ORG. However, plant height was not affected by the farming systems and no significant differences were observed.

4.5 Discussion

The objective of the present manuscript was to compare the effects of organic and conventional farming systems on the microbial biomass, activity and composition as well as soil CO_2 emission and readily available nitrogen forms into the soil in a long-term experiment in Tuscany, Italy. The implementation of environmentally friendly agricultural policies has increased the need to compare some agricultural aspects of conventional and organic farming systems (García-Ruiz et al., 2008). This type of study is strongly needed to better understand

Table 6: *Yields and morphological parameters of sunflower in ORG and CON systems*

	Units	**ORG**	**CON**	
Yield	t ha^{-1}	1.41 (±0.66)	2.10 (±0.89)	***
Flowers diameter	cm	6.35 (±3.5)	14.7 (±6.6)	***
Number of seeds per flower	–	377.1 (±205.1)	873.1 (±393.1)	***
Seeds weight per flower	gr	19.9 (±10.9)	43.6 (±19.7)	***
Plant height	cm	116.0 (±46.1)	143.4 (±41.2)	NS

Note: Standard deviations of data are in brackets. Statistical difference according to the ANOVA analysis are reported: NS, not significant; ***, significant at probability level $p<0.001$.

the role of organic farming to improve soil quality and benefit the environment. A recent study (Zani et al., 2022) reported a significant potential of organic farming to improve soil quality (fertility, biodiversity, C and nutrients stock). However, due to the complexity of the soil system, there is still a lack of scientific knowledge to maintain soil productivity and biodiversity in the long term. The novelty of this study lies in the essence of the MoLTE experiment itself; in fact, long-term experiments can give important information to assess soil fertility in a long-term perspective. Backed by these considerations, we have measured different parameters relating to soil microbial community, GHG emissions, N content and sunflower production at various intervals corresponding to the main sunflower phases (Table 2). The greatest microbial biomass expressed as DNA yield was found at t18, as well as the greatest amounts of bacterial and fungal sequences evaluated by 16S and 18S sequences in qPCR, for both the ORG and CON. Reasonably, this was the consequence of fertilization carried out at the beginning of the experiment (t0) in ORG and CON, respectively. The ammonia-oxidizing bacteria, monitored by *amo*A qPCR, increased significantly between t83 and t104 in CON, only. This delay was expected, due to the slow growth rate of the AOB population compared with other fast-growing bacteria and fungi, but also the soil N content and its availability has to be considered. In fact, the amount of *amo*A gene copies was more abundant in CON differently from the bacterial and fungal sequences. As regard to the N content and release, it is known that chemical fertilizers used in conventional farming, such as ammonium nitrate and urea, result in a significant accumulation of ammonium, easily available for AOB, and nitrate (Jia & Conrad, 2009). After all, the ability of many ammonia oxidizers to hydrolyse urea is well known and this fertilizer has been found to stimulate autotrophic nitrification in soil, independently from pH (Burton & Prosser, 2001). Moreover, in CON, ammonia oxidizers during the t52-t104 interval were more abundant than in ORG while, after this time, their copy number decreased to almost the same amount at t0. CO_2 emissions were not constant during the experiment in either of the two managements. In fact, it was minimal at the beginning and at the end of the full growing season, when presumably the soil microbial communities existed under steady-state-like conditions. Interestingly, only in ORG, were emissions greatest between t52 and t104, during which sunflower stem elongation and flowering occurred. This could be a result of the intense metabolic activity occurring during the vegetative phase in the period of intense stimulation and interactions among plant roots and microorganisms (Alami et al., 2000), and we could refer to this period as the *hot moment* of the microflora in the soil systems (Kuzyakov & Blagodatskaya, 2015). We could argue that during this period there were more inputs of labile organics in soil deriving from root exudates and decomposing materials, but also other internal triggering signals as auto-inducer molecules secreted by the microbial communities themselves able to wake them up from dormancy to activity (Raffa et al., 2005). We also considered biochemical and molecular data for the full period of the sunflower cycle (t0-138 days) to provide a global vision of the microflora, its activity, soil N emissions and RMO-N. The soil total DNA yield, corresponding to the microbial biomass, was less in CON than in the ORG. Applying the ratio of soil CO_2 emissions to the total DNA yield and also to bacterial (16S) and fungal (18S) gene copies separately, it was possible to distinguish the physiological activity, indicated here as $mqCO_2$, of the soil microbial biomass as a whole, and of the bacterial and fungal communities distinctly. The latter two parameters constitute a new methodological aspect that we have applied in this work. Results showed that the microbial biomass was more active and abundant in ORG; despite a lower amount, fungi were more active than bacteria, both in ORG and in CON farming. The lower $mqCO_2$ of bacteria may indicate that they could belong more to maintenance strategists than to resource acquisition strategists (Ramin & Allison, 2019). Still considering the full period, ammonia oxidizers represented 0.1% and 0.5% of the bacterial community in ORG and CON soils, respectively. The positive correlation between soil NO_3^+ concentration and AOB was indicative of ammonia oxidation activity (i.e. end product), supporting the soil mineral-N associations with AOB populations and the easier availability of N content of the mineral fertilizers used in conventional agricultural systems (Tao et al., 2017). Moreover, we decided to conduct a preliminary study, focusing on the bacterial community, by 16S rRNA gene sequencing, on the basis of the essentiality of microbial diversity for soil. We, therefore, examined *alpha* and *beta* diversity and related indices comparing the two types of agricultural managements. The Venn diagram showed that a large proportion of bacteria was shared between the two managements and these might be considered a 'core microbiome' (Estendorfer et al., 2020) composed of poorly characterized microbes and presumably present in many soils, although not equally abundant. The presence of unique OTUs in ORG and CON samples may be due to selective soil properties deriving from different managements. The results of Chao1 and species richness clearly showed that ORG treatment significantly increased the bacterial richness. This may be due also to the green manuring adopted for the ORG management, based on a grass-legume mixture, more easily decomposable and known for greater N mineralization, as well as having a positive influence on the physical and chemical properties of the soil (Fageria, 2007). The variations in *beta* diversity and relative abundance at phylum and family level were not significantly affected by the management practices, other than for the phylum *Gemmatimonadetes* (family *Gemmatimonadaceae*); in fact, they were significantly greater in CON than in ORG soil samples. This phylum has a wide distribution in soil systems and it is frequently detected in environmental 16S rRNA gene libraries, representing the top nine phyla in soils, comprising 2% of soil bacterial communities. Since such microorganisms have only recently been studied, little is still known about their role in agricultural systems, other than that they are particularly suitable for arid environments. Their constant presence suggests a versatile metabolism that allows to survive well in soil and perhaps to withstand the impacts of global warming (DeBruyn et al., 2011; Douglas Madison et al., 2021; Orr et al., 2015). *Cyanobacteria*, *Actinobacteria* and *Proteobacteria* were the most abundant phyla, contributing more than others to the differences between 16S rRNA gene pools in the two management systems as shown by the SIMPER test. The presence of these three phyla, to which many generalist bacteria suitable for different environmental conditions belong, was of some importance. The sunflower crop could have influenced the soil microbial community through its root exudates (Tejeda-Agredano et al., 2013), but this aspect was not taken into consideration in this study. We are aware that these are preliminary results and that further metagenomic studies will have to be done, but, nevertheless, these data could be indicative of the microbial diversity in the considered soil under long-term managements. The transfer of nitrate from cultivated soil to groundwater is another environmental concern linked to agriculture. In this sense, organic farming has come into focus as a possible way to reduce nitrate leaching from arable land (Kirchmann & Bergström, 2001). In this study, the soil NH_4^+ and NO_2^- contents in the t0-138 period were not significantly different between ORG and CON, while NO_3^+ was 30% less in ORG. This latter result, although referring to 1 year only, is in line with literature where NO_3^+ concentrations were greater in conventional than in organic plots (Benoit et al., 2015; Kramer et al., 2006). The greater RMO-N concentration in ORG could be caused by the differences in fertilization methods. However, it cannot be excluded that, as soon as the conditions would be favourable for AOB in organic systems, the soil RMO-N content could increase. Nevertheless, despite greater soil microbial community development and activity, ORG sunflower yield was about 33% less than CON, confirming previous experimental evidence (Mazzoncini et al., 2006; Seufert et al., 2012). However, both CON and ORG produced smaller yields compared with the regional average production and this was mainly due to the dry season that occurred in 2018 (Figure 1) and the absence of an irrigation system. Yield gap is the main issue of organic farming, and some authors share the concern that organic agriculture may need an increased cultivated area due to reduced yields (Tuomisto et al., 2012; Villanueva-Rey et al., 2014). Indeed, the increase in resource use efficiency per unit area of land is a key point for the improvement of organic farming performance, although research studies have not yet reached a unique answer to this point. For reducing yield gap, a great challenge for future research in organic farming is to deepen the knowledge on weed control, phosphorus (P) availability in soils, stimulation of soil microbial biomass and use of selected crop varieties able to grow on low-input farming systems. Our results also showed that differences in N rate due to different fertilization in ORG and CON significantly affected morphological parameters such as flower diameter, number of seeds per flower and seed weight per flower, as found by other authors (Abdel-Motagally & Osman, 2010, 2010; Tripathi et al., 2003). Nevertheless, plant height seems to be not affected by farming systems and this is probably due to the physiology of the crop that consumed the main part of soil resources for the vegetative growth and, in ORG, a nutrient lack for grain differentiation. Sustainability assessment requires a comprehensive perspective that accounts for the interrelationships between the technical, environmental, social, economic and political aspects (Pacini et al., 2003). In this study, we did not consider the impacts of ORG and CON on financial and food quality aspects, which instead hold considerable importance in terms of overall sustainability of farming systems. Indeed, Var. Toscana chosen for ORG is a sunflower variety used as seed for human consumption; they are consumed as snacks and obtain considerably higher prices on the market than Var. LG50.525 cropped for CON. Hence, smaller yields in organic farming can nevertheless produce greater revenues than conventional agriculture. From a health perspective, organic products can provide a valid tool for sustainable food consumption (European Commission, 2020). However, on a global scale, the consumer education is crucial to discriminate that there is no low or high price but a fair or unfair price for a healthier food chain production.

4.6 Conclusion

Organic agriculture is a holistic production management system, which promotes and enhances agroecosystem health, biodiversity and biological cycles. Thus, it becomes crucial to compare the effects of long-term organic and conventional systems on soil indicators. Our results showed that, during the year of the study, bacteria and fungi were more abundant, active and diverse in soil under organic farming despite the lesser N inputs, while the sunflower yield was significantly less in organic than in conventional farming. However, the benefits of organic farming should be considered in the overall context of the environmental-friendly production that includes social, economic and environmental aspects. In this sense, the scientific community can have an important role in promoting low-input farming systems to farmers, policy makers and citizens.

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4.6.0.2 Conflict of Interest

The authors declare no conflict of interest.

4.6.0.3 Author contribution

Margherita Santoni: Conceptualization; data curation; formal analysis; investigation; methodology; writing-review & editing.
Leonardo Verdi: Conceptualization; data curation; methodology; writing-review & editing.
Shamina Imran Pathan: sequencing data curation; investigation.
Marco Napoli: data curation; methodology; writing-review & editing.
Anna Dalla Marta: Conceptualization; funding acquisition; project administration; writing-review & editing.
Francesca Romana Dani: Conceptualization; funding acquisition; project administration; writing-review & editing.
Gaio Cesare Pacini: Project Manager of the MoLTE, Montepaldi Long Term Experiment.
Maria Teresa Ceccherini: Conceptualization; funding acquisition; project administration; supervision; writing-review & editing.

4.6.0.4 Data Availability Statement

The data that support the findings of this study are available from the corresponding author (Dr. Verdi). The authors are pleased to share the data upon request.

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5 A Review of Scientific Research on Biodynamic Agriculture

Margherita Santoni[1], Lorenzo Ferretti[1], Paola Migliorini[2], Concetta Vazzana[1], Gaio Cesare Pacini[1]

[1] Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali (DAGRI) - University of Florence (Italy)
[2] University of Gastronomic Sciences of Pollenzo, Bra, Italy

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5.1 Abstract

Biodynamic agriculture (BD agriculture) was presented as an alternative form of agriculture by the philosopher Rudolf Steiner and is nowadays considered one of the forms of organic agriculture. The objective of the present manuscript is to critically review international scientific literature on biodynamic agriculture as published in highly ranked journals and to assess its performance. This review was based on a structured literature survey of peer-reviewed journals indexed on the Web of Science™ (WoS) Core Collection database carried out from 1985 until 2018. We found 147 publications of studies in journals with an impact factor. Of these, 93 focused on biodynamic agricultural practices, 26 on the sustainability of the biodynamic method, and 28 on the food quality of biodynamic products. The results of the literature review showed that the BD method enhances soil quality and biodiversity. Instead, further efforts are needed to implement knowledge on the socio-economic sustainability and food quality aspects of BD products. One particularly promising topic of research consists in the assessment of microbial activity and the potential that microbiomes have in BD farms to enhance soil fertility and human health following the One Health approach. Moreover, it is critical that such subjects be investigated using a systemic approach. We conclude that BD agriculture could provide benefits for the environment and that further efforts should be made with research and innovation activities to provide additional information to farmers, policy makers, and stakeholders regarding this type of organic agriculture.

Keywords: literature review, biodynamic agriculture, organic agriculture, agricultural practices, sustainability, food quality

5.2 Introduction

Biodynamic agriculture (BD agriculture) was presented as an alternative form of agriculture by the philosopher Rudolf Steiner (Steiner 1924) and is nowadays considered one of the forms of organic agriculture. The BD method is based on a closed production system that aims to reproduce an agroecological model focused on a reduction of energy consumption and capable of achieving high levels of environmental efficiency. The method has been institutionalized by the international certification label Demeter (Döring et al. 2015). As reported by Willer et al. (2020), since the turn of the millennium, Demeter-certified farms have grown significantly in number (more than 5900 farms in June 2019), and the certified surface area has almost doubled to over 200,000 ha in 63 countries. Germany has the largest BD area (34% of the world total), followed by Australia (20%), and France (6%) (Paull et al. 2020). In total, around 15,000 ha of the Demeter-certified area are biodynamic vineyards, with around 760 BD wineries in Europe, led by France with 375 wineries (Willer et al. 2020). In comparison to the global total of 71.5 million certified organic hectares, BD farming represents a small niche as it covers only 0.35% of the land in question (Paull and Hennig 2020). BD and organic agriculture share most principles and rules; however, Demeter's production rules include restrictions on many organic farming practices in order to strengthen the multifunctional role of the farm. Demeter-certified farms fully comply with organic agriculture rules but impose additional obligations. The main differences between Demeter and organic production rules as defined by the International Federation of Organic Agriculture Movements (IFOAM) concern the use of specific preparations applied to crops or soil in very small amounts Table 1, the obligation to leave 10% of the total farm area available for ecological infrastructures, and the obligation to rear animals on the farm (0.2 livestock units per hectare). While the use of preparations has always been compulsory, the minimum ecological infrastructure areas rule entered into force recently, and the constraint on animals currently applies only to Italian farms (Demeter Associazione Italia). However, although in the past, only preparations were normed, it has always been standard practice for BD farms to promote biodiversity and rear animals within the farm.

The hypothesis at issue is whether BD methods possess the capacity to support optimum performances in terms of agroecosystems and human health. In recent decades, international research has examined BD agriculture to assess whether the BD method affects ecosystems, crops, and products. Even though the BD method is not in widespread use around the world, these aspects, combined with potential impacts on biodiversity and overall sustainability, make the BD method an interesting option for agroecosystem management. The number of scientific studies investigating BD agriculture is restricted when compared to those investigating organic agriculture, which has attracted considerable interest in the scientific community. The first studies specifically focusing on the BD method were carried out between the end of the 1980s and the beginning of the 1990s, while the most recent peer-reviewed research into BD agriculture was published by Turinek et al. in 2009. On the basis of these considerations, the objective of this paper is to critically review international scientific literature on BD agriculture as published in highly ranked journals and to assess its performance, as well as to detect any lack of knowledge on relevant issues in agriculture, if any exist. In the concluding section, the results obtained are discussed in the context of the development of sustainable agriculture, with some specific suggestions for further development of BD research.

5.3 Materials and Method

A review of international scientific literature on BD agriculture was conducted with specific reference to highly ranked journals. The review was based on a structured literature survey of peer-reviewed journals indexed on the Web of Science™ (WoS) Core Collection database carried out for all years from 1985 until 2018. All possible combinations of the terms "biodynamic," "bio-dynamic," "agriculture," and "farming" were used for the literature search and no other search terms were considered as we wanted to focus exclusively on studies aimed specifically at BD agriculture. Conference proceedings were excluded from the search. The whole set of WoS categories were considered. The document types considered were articles and reviews published in English in scientific journals

Table 1: *List of the main biodynamic preparations (Masson, 2009)*

Preparation number	Main ingredient
500	Cow manure
500P	Preparation 500 with 502–507
501	Silica
502	Yarrow flowers (*Achillea millefolium*)
503	Camomile flowers (*Matricaria recutia*)
504	Stinging nettle shoots (*Urtica dioica*)
505	Oak bark (*Quercus robur*)
506	Dandelion flowers (*Taraxacum officinale*)
507	Valerian extract (*Valeriana officinalis*)
Compost	Cow manure with preparation 502 to 507

with impact factor. The references were exported to our database; double entries and material not related to BD agriculture were excluded. Statistical analyses were conducted on accumulation of BD agriculture publication over time and on geographical distribution utilizing R statistical software version 4.0.3 (R Core Team 2020) and one of its libraries (Wickham 2011). Articles were then grouped based on their correspondence to three topics: (a) biodynamic agricultural practices, (b) sustainability of the biodynamic method, and (c) food quality of biodynamic products. The relevance of targeted journals of BD agriculture studies was considered in terms of impact factor (IF) by dividing the publications into three categories: publications in journals with (i) $0 < \text{IF} < 1$, (ii) $1 < \text{IF} < 2$, and (iii) $\text{IF} > 2$. For each journal, the Five-Year Journal Impact Factor™ referring to 2018 (source: Journal Citation Report™) was considered and was taken directly from the Journal information section of Web of Science™. Additionally, we selected first-quartile articles from among those belonging to the third IF category ($\text{IF} > 2$). Our qualitative remarks referred to the last category. To compare the extent of studies carried out of BD agriculture with those conducted on Organic and Integrated Agriculture, we used more selective entries and counted total publications of literature searches for three groups of topics:

i. "Biodynamic Agriculture," "Biodynamic Farming," "Bio-dynamic Agriculture," "Bio-dynamic Farming;"
ii. "Organic Agriculture," "Organic Farming and
iii. "Integrated Agriculture," "Integrated Farming," "Integrated Crop Management," "Integrated Pest Management".

5.4 Results

The number of articles on BD agriculture published between 1985 and 2017 is shown in Figure 1. Publication of research in journals with impact factor started recently, i.e., 1990, for a total amount of 147 articles, of which 87 were published in the last decade. This means that in 33 years of potential publication, less than five articles per year have been published. When we compare the 147 publications focusing on BD agriculture with the number referring to Organic Agriculture (5498) and Integrated Agriculture (6676), we deduct that the research effort into BD agriculture carried out is indeed at an early stage of development. Of the total of 147 articles reporting to a broad extent studies on BD agriculture, 82 resulted in IF > 2 and 68 (46% of the total) belonged to the first quartile of the corresponding WoS category. The worldwide geographical distribution and focus on the Mediterranean area of articles published on peer-reviewed journals indexed on the Web of Science™ (WoS) Core Collection database from 1985 until 2018 are reported in Figure 2. Most of the studies in the articles published on BD agriculture were carried out by institutions located in Europe: 54% were conducted in North and Central Europe (Germany, Sweden, Switzerland, Netherlands, UK, Ireland, Lithuania, Czech Republic, and Austria), 12% in Italy, and 6% in other Mediterranean countries (Spain, Slovenia, and Tunisia); 12% of research was carried out in Oceania (Australia, New Zealand), 7% in North America (USA, Canada), 6% in Asia (India, Philippines), and 3% in South America (Brazil, Venezuela). The amounts of articles published on three major themes regarding BD agriculture, i.e., biodynamic agricultural practices (a), sustainability of the biodynamic method (b), and food quality of biodynamic products (c), are shown in Figure 3. The number of articles referring to BD agriculture practices, sustainability, and food quality amounted to 93, 26, and 28, respectively (i.e., 63.3, 17.7, and 19.0%). Moreover, sustainability and food quality articles never exceeded two publications per year, with many years featuring no publications at all. Studies regarding food quality are exclusively recent, with the first publication in IF journals in 2004.

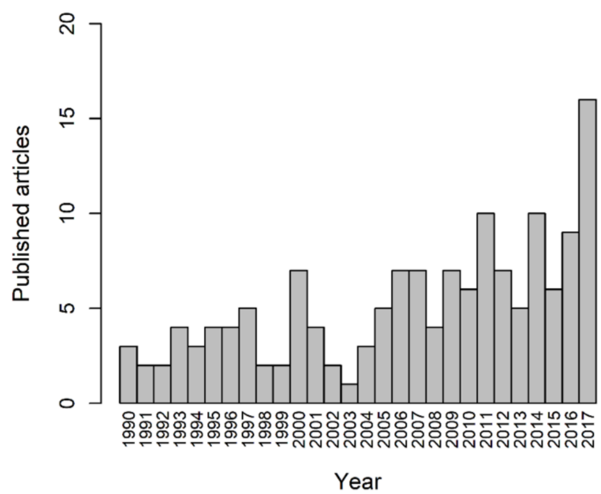

Figure 1: *Total number of articles on biodynamic agriculture published in peer-reviewed journals indexed on the Web of Science™ (WoS) Core Collection database from 1985 until 2017. Articles published before 1990 were not found in the database*

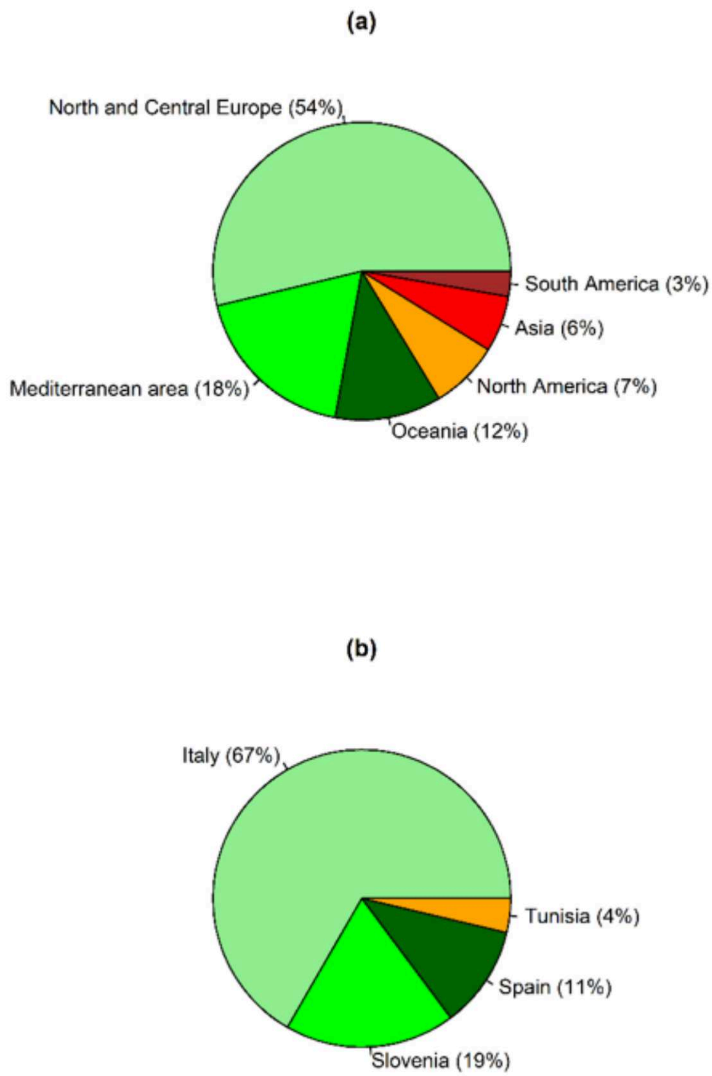

Figure 2: *Worldwide geographical distribution (a) and focus on the Mediterranean area (b) of the articles published in peer-reviewed journals indexed on the Web of Science (WoS) Core Collection database from 1985 until 2017*

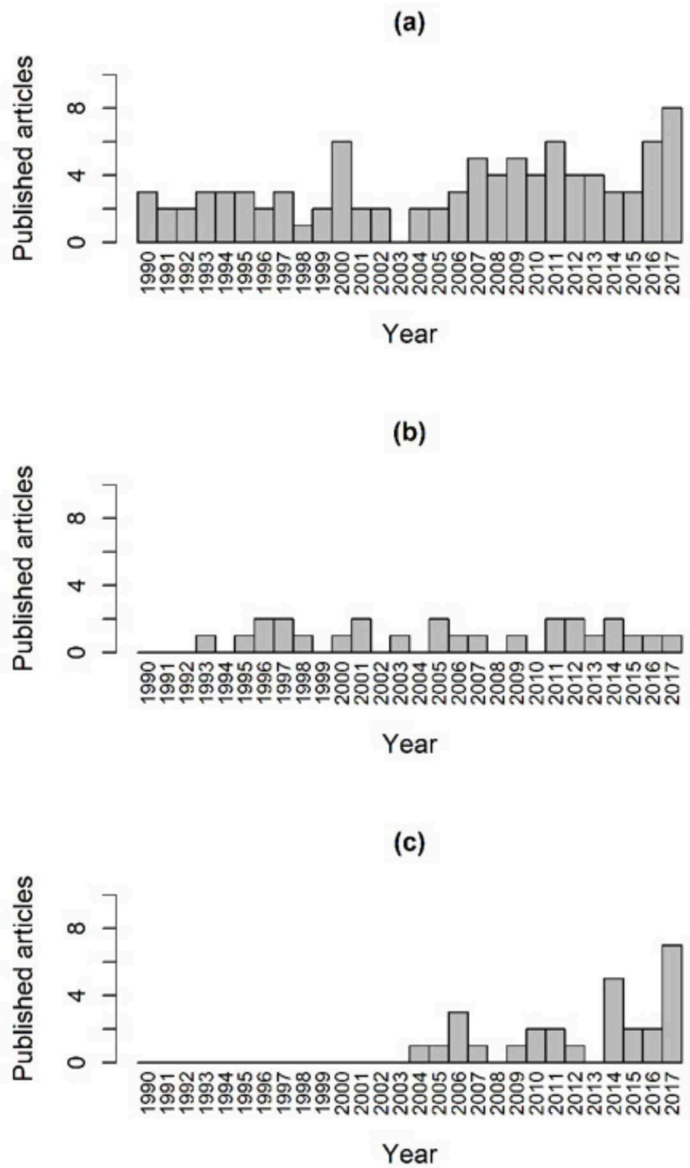

Figure 3: *Number of the articles published in peer-reviewed journals indexed on the Web of Science.™ (WoS) Core Collection database from 1985 until 2017 grouped by three topics, i.e., biodynamic agricultural practices (a), sustainability of the biodynamic method (b), and food quality of biodynamic product (c)*

Table 2: *A selection of the most informative publications on the impacts of biodynamic practices*

Location of the trial	Trial description	Trial duration	Years of experiment	Size of experimental plots or samples	Parameter to assess BD[1] practices	References
Therwil-1, Switzerland	Long-term field trial ("DOK" trial)—system comparison between biodynamic, organic, two conventional and one control (unfertilized) in arable cropping systems	1978–the present day	21 years	100 m^2	Soil aggregate stability, soil pH, stable organic matter formation, soil calcium and magnesium, microbial and faunal biomass, grain yield, energy use and efficiency	Mäder et al. (2002)
				100 m^2	Soil organic carbon, soil pH, total soil nitrogen, soil microbial biomass, soil microbial activity, soil dehydrogenase activity, soil basal respiration	Fließbach et al. (2007)
			1 year	100 m^2	Weed seedbank abundance, diversity, and community composition	Rotchés-Ribalta et al. (2017)

Table 2 – *(continued)*

Location of the trial	Trial description	Trial duration	Years of experiment	Size of experimental plots or samples	Parameter to assess BD[1] practices	References
Therwil-2, Switzerland	Long-term field trial ("DOK" trial)-system comparison between biodynamic, organic, two conventional systems using mineral fertilizers and farmyard manure at two fertilization intensities (50% of standard fertilization and standard fertilization) in winter wheat (Triticum aestivum L.)	1978-the present day	7 years	100 m^2	Crop yields, baking quality parameters, nitrogen use efficiency, effect of maize and potatoes as preceding crops	Mayer et al. (2015)
Frick, Switzerland	Long-term field trial-effects of reduced tillage, organic fertilization strategies, and biodynamic preparations on organic grassland, pastures, and arable crop	2002-the present day	6 years	144 m^2	Soil organic carbon, soil microbial biomass, soil microbial activity, soil nutrients, soil nutrient budgets	Gadermaier et al. (2012)
Baden-Württemberg, Germany	10 organic horticultural farms (5 biodynamic and 5 organic)	n.a.[3]	3 years	Soil samples	Plant available phosphorus, soil potassium, soil organic carbon, soil pH, soil salinity	Zikeli et al. (2017)
Geisenheim, Germany	Long-term field trial—system comparison between integrated, organic, and biodynamic vineyards	2006–the present day	3 years	216 m^2	Plant growth response, physiological performance, yield, soil nutrient status, disease incidence, wine grape quality	Döring et al. (2015)

Table 2 – *(continued)*

Location of the trial	Trial description	Trial duration	Years of experiment	Size of experimental plots or samples	Parameter to assess BD[1] practices	References
Wairau valley, New Zealand	Field crop trial comparison between six conventional and six biodynamic vineyards	n.a.[3]	1 year	Bark, fruit, and soil samples	Fungal diversity across vineyard habitats (bark, fruit, soil)	Morrison-Whittle et al. (2017)
Tebano, Italy	Long-term field trial—system comparison trial between organic and biodynamic grapevines	From 2008 to 2013	3 years	84 m^2	Plant physiological responses, characterization of biodynamic preparations	Botelho et al. (2016)
Sfax, Tunisia	Biodynamic olivegrowing farm	The farm has been managed biodynamically for 15 years at the time of publication	1 year	Soil samples	Bacillus spp. abundance and pathogenicity to lepidopterans and coleopterans	Blibech et al. (2012)
Darmstadt, Germany	Long-term field trial—comparison between three different fertilizers: inorganic, composted farmyard manure, and composted farmyard manure with the addition of BD[1] compost and preparations in arable cropping systems	1980–the present day	1 year	25 m^2	Soil microbial community composition in terms of AMF[2] and saprotrophic fungal biomass	Faust et al. (2017)

Table 2 – *(continued)*

Location of the trial	Trial description	Trial duration	Years of experiment	Size of experimental plots or samples	Parameter to assess BD[1] practices	References
Hopland, California (USA)	Composting of a grape pomace and manure mixture with and without BD[1] compost preparations. Water extracts of finished composts were then used to fertigate wheat seedlings (*Triticum aestivum L.*), with and without added inorganic fertilizer	n.a.[3]	2 years	Compost and wheat seedlings samples	Chemical, physical, and biological analyses of the compost. Growth response of wheat seedlings to aqueous compost extracts	Reeve et al. (2010)
Lopez Island, Washington State (USA)	Treatment comparison between lime, BD[1] preparations and an untreated control on permanent pasture	The farm has been managed organically for over 38 years at the time of publication	2 years	225.7 m^2	Forage yield and quality, soil pH, total soil C and N, soil microbial activity, farm economic and social sustainability	Reeve et al. (2011)
Rome, Reggio Emilia and Bolzano, Italy	Different commercial samples of BD[1] preparation 500 from three Italian producers	n.a.[3]	2 years	BD[1] preparation samples	Microbiological characterization and biological activities of preparation 500	Giannattasio et al. (2013)

[1]Biodynamic
[2]Arbuscular mycorrhizal fungal
[3]Not applicable

Table 3: *A selection of most the informative publications on sustainability of the biodynamic method*

Sustainability domain	Location of the trial	Trial description	Length/years of experiment	Assessment method	References
Environment	Policoro, Italy	Long-term field trial-system comparison between two integrated and one biodynamic apricot orchard	20 years	Life cycle assessment (LCA), energy analysis (EA)	Pergola et al. (2017)
	Leiro and San Amaro, Spain	Field trial—system comparison between biodynamic and conventional vineyards	2 years	Life cycle impact assessment (LCIA), land competition (LC), human labor (HL)	Villanueva-Rey et al. (2014)
	Pivola, Slovenia	Long-term field trial—system comparison between conventional, integrated, organic, and biodynamic wheat and spelt production	3 years	Ecological footprint, overall footprint per unit, sustainable process index, ecological efficiency of production	Bavec et al. (2012)
	Therwil and Burgrain, Switzerland	Two long-term field trials-system comparison between biodynamic, organic, and conventional/integrated systems (“DOK” trial); integrated intensive, integrated extensive, and organic systems (“Burgrain” trial) in arable cropping and forage production systems	DOK trial: 14 years Burgrain trial: 5 years	Swiss agricultural life cycle assessment, life cycle inventory, life cycle impact assessment	Nemecek et al. (2011a)

Table 3 – *(continued)*

Sustainability domain	Location of the trial	Trial description	Length/years of experiment	Assessment method	References
	Therwil, Burgrain and Zollikofen, Switzerland	Three long-term field trials-system comparison between biodynamic, organic, and conventional/integrated systems ("DOK" trial); integrated intensive, integrated extensive, and organic systems ("Burgrain" trial); conventional plowing and no-till soil cultivation systems ("Oberacker" trial) in arable cropping and forage production systems	DOK trial: 14 years Burgrain trial: 5 years Oberacker trial: 6 years	Life cycle assessment (LCA)	Nemecek et al. (2011b)
Economic	North Island of New Zealand	16 biodynamic and conventional farms including market garden (vegetables), pip fruit (apples and pears), citrus, grain, livestock (sheep and beef), and dairy	4 years Farms economic profitability through the MAF[1]	Reganold et al. (1993)	
	Madhya Pradesh, India	Field trial system comparison between biodynamic, organic, and conventional cotton-soybean-wheat crop rotations	4 years	Agronomic, economic, and ecological performance, gross margin of cotton, soybean, and wheat	Forster et al. (2013)
Social	USA	System comparison between biodynamic, organic, and conventional agriculture	n.a[2]	Bruno Latour's circulatory model	Ingram (2007)
	Ireland	Interview with six biodynamic farmers	n.a[2]-the interview was done in 2001	Social analysis	McMahon (2005)

[1]Models used by the New Zealand Ministry of Agriculture and Fisheries (1987–1991)
[2]Not applicable

Table 4: *A selection of the most informative publications on food quality of biodynamic products*

Location of the trial	Trial description	Products	Years of product harvest	Size of experimental plots or samples	Parameters for assessing food quality	References
Therwil, Switzerland	Long-term field trial ("DOK" trial)-system comparison between biodynamic, organic, two conventional and one control (unfertilized) systems	Wheat grains (*Triticum aestivum L.*)	2003	Samples	Sugars, sugar alcohols, amino acids, organic acids	Zörb et al. (2006)
Lenart, Slovenia	Field trial comparison between integrated, organic, biodynamic, and control (unfertilized) systems	Rapeseed (*Brassica napus L. "Siska"*) seeds	2009/2010 and 2011/2012	72 m^2	Water, protein, oil, glucosinolate, fatty acid composition	Turinek et al. (2016)
Florence, Italy	Field trial comparison between biodynamic, and conventional systems under water stress or standard conditions	Chicory (*Cichorium intybus L.*)	2006/2007	Samples	Polyphenol content, antiradical activity	Heimler et al. (2009)

Table 4 – *(continued)*

Location of the trial	Trial description	Products	Years of product harvest	Size of experimental plots or samples	Parameters for assessing food quality	References
Darmstadt, Germany	Field trial comparison between conventional, organic, and biodynamic systems	Batavia lettuce (*Lactuca sativa L. ssp. acephala L.*)	2008	6 m^2	Yield, polyphenol content (flavonoids, anthocyans, hydroxycinnamic acids), antiradical activity	Heimler et al. (2012)
Pivola, Slovenia	Long-term field trial-system comparison between conventional, integrated, organic, biodynamic, and control (unfertilized) systems	Red beet (*Beta vulgaris L.* ssp. vulgaris Rote Kugel)	2009	70 m^2	Sugar, organic acid, total phenolic content, antioxidative activity	Bavec et al. (2010)
14 states in Brazil and Europe	Organic, biodynamic, and conventional products	Purple grape juices	n.a.[1]	Samples	Volatile organic compounds	Granato et al. (2015)
Literature review	Organic, biodynamic, and conventional products	Purple grape juices	1998–2016	n.a.[1]	Chemical composition, functional properties	Granato et al. (2016)

Table 4 – *(continued)*

Location of the trial	Trial description	Products	Years of product harvest	Size of experimental plots or samples	Parameters for assessing food quality	References
Alghero, Mamoiada, Mores, and Santadi, Italy	Field trial comparison between three conventional and one biodynamic systems	Purple grape juices from cultivar "Cannonau"	2015	Samples	Microbial diversity on wine must	Mezzasalma et al. (2017)
Gelderland and Friesland/Groningen, Netherlands	Comparison between three organic, three biodynamic, and 24 conventional farms	Cow milk	2011	Samples	Fat content, protein, lactose, urea, unsaturated fatty acid, milk freezing point depression	Capuano et al. (2014a)
					Fatty acid profiling, chemometric modelling	Capuano et al. (2014b)

[1] Not applicable

5.4.1 Result of the Literature Survey on Biodynamic Agricultural Practices

It is not easy to draw generic, globally valid conclusions on the impacts of BD agricultural practices based on such a small number of publications (93). However, a few tentative considerations can be made based on consolidated outcomes published in important publications since the 1990s, although only in reference to specific pedo-climatic and production conditions.

There are 42 articles within the "BD practices" topic belonging to the first quartile of the corresponding WoS category and with IF > 2. Articles presenting generic results as broadly as possible applicable to corresponding production systems (i.e., arable cropping and horticulture, viticulture, and olive tree cropping) were selected for further analysis. Our concern was to cover as many production systems as possible and consider those publications that produced generically applicable results. The selection of most informative publications on the impacts of BD practices is shown in Table 2 together with geographical location of the trials, trial description and duration, duration of the single experiments, size of experimental plots or samples, and parameters employed to assess the impacts of BD practices. Most articles included in the BD practices group refer to aspects of soil quality. As reported by Mader et al. (2002), BD practices, which primarily make use of preparation 500, improve the overall soil quality. Reganold et al. (1993, Table 3), comparing 16 BD and conventional farms in New Zealand, found that BD farms had better soil quality than conventional ones. In BD farms, significantly higher organic matter content and microbial activity, earthworm abundance and infiltration rate, better soil structure, aeration and drainage, and lower bulk density as well as thicker topsoil were found. Several selected articles focus on outcomes from the well-known, 40-year-old DOK trial, which were published between 1993 and 2017 in highly ranked journals like "Science." These articles report on long-term comparisons between biodynamic, organic, and two conventional arable cropping systems. Based on the outcomes of the experiment in Therwil, Switzerland (Table 2, Therwil-1), the authors conclude that organically manured, legume-based crop rotations utilizing organic fertilizers from the farm itself are a realistic alternative to conventional farming systems. As regards soil aggregate stability, soil pH, stable organic matter formation, soil calcium and magnesium, microbial and faunal biomass (earthworm, carabids, staphylinids, and spiders), the BD system demonstrated the potential to be superior, under given circumstances, even as compared to the organic system (Mader et al. 2002). In a later study in the DOK trial (Table 2, Therwil-1), Fliebach et al. (2007) found that soil pH, total soil N, and soil organic carbon are higher in BD systems as compared to conventional systems. In addition, soil microbial biomass, soil organic matter for microbial biomass establishment, and dehydrogenase activity are higher in BD systems, indicating better soil quality in BD systems. In this article, it was also found that the metabolic quotient for CO2 (qCO2), which summarizes microbial carbon utilization, was higher in conventional as compared to BD soils, suggesting a higher maintenance requirement for microbial biomass in conventional soils.

However, as regards soil microbial biomass C/N ratio (Cmic-to-Nmic), which is an indicator of biological soil fertility (Sparling 1992; Stockfisch et al. 1999), a treatment with compost and BD preparations reported lower performances as compared to a conventional manured system. The authors were unable to say whether this effect was caused by composting or by the BD preparations. This trend was not confirmed by Gadermaier et al. (2012) who stated that BD preparations increased the Cmic-to-Nmic in the Frick long-term experiment in Switzerland (Table 2). From these studies, some additional conclusions can be drawn in terms of impact on agroecosystem biodiversity. The research carried out by Mader et al. (2002) stated that BD preparations positively impact biodiversity. Moreover, Rotchés-Ribalta et al. (2017), in a study carried out in the DOK trial (Table 2, Therwil-1), found that weed seedbank abundance, diversity, and community composition were higher in the BD systems as compared with those of the conventional systems. They also found that high inputs of mineral fertilizers selected for more nitrophilous species, while herbicide applications selected against herbicide-susceptible species. Crop yields are influenced by agricultural practices, and much research has focused on studying the differences between organic and conventional agriculture. However, only a few studies take into consideration BD agriculture. Among them, studies regarding arable cropping systems confirm that mean crop yields in BD farming are lower than those of conventional systems (Mader et al. 2002; Mayer et al. 2015). While this is a common outcome of much research comparing yields of BD agriculture and also organic farming in many productive sectors, it is worth mentioning that higher yields are more often than not a result of higher input use which comes with a monetary but also with an energy and an ecological cost, as is more extensively remarked in the section below. Yield differences between organic and BD farming were surveyed by Zikeli et al. (2017) in a study of ten BD and organic greenhouses in Southern Germany. In this study, the BD farms had statistically significant higher yields in tomatoes and cucumbers as compared to the organic farms. Despite higher yields from BD farms, authors found strong imbalances between organic and BD farms as regards nutrient flows, with high average surpluses for N, P, S, Ca, and Na, which could lead to risks of increased soil alkalinity and salinity. Moreover, BD farms showed a lower N use efficiency (NUE) and significantly lower concentrations of soil available P. These imbalances were also confirmed by Mayer et al. (2015) in a previous study in the DOK trial (Table 2, Therwil-2). In this study, the conventional farming system at half standard fertilization level had a better NUE than organic and BD systems. Furthermore, low organic fertilizer inputs lead to degradation of soil quality in organic as well as in conventional systems. The results showed that fertilization strategies in organic and BD farming systems are a focal point for developing new strategies to avoid long-term nutrient imbalances. BD practices have been mainly tested in vineyard production systems. This is because in recent years, many wine farms have decided to convert to BD agriculture (Demeter and BDA Certification 2020). A recent study involving a long-term trial in vineyards found that the organic and the BD treatments showed higher soil nitrogen levels, which had been successfully ensured through cover crop management and compost addition (Doring et al. 2015). However, magnesium content in leaf tissues, an important parameter required for chlorophyll composition, was found to be significantly higher in the integrated treatment, while phosphorous and potassium contents did not show any relevant differences. This is in line with the findings of an article published in Nature Scientific Reports, which stated that 10 years of different management practices had not caused any major shifts in terms of physicochemical soil parameters, and the only parameter exhibiting relevant differences was magnesium, which was found to be lower in BD systems (Hendgen et al. 2018). However, in terms of microbial activity, soil under integrated management had a significantly reduced bacterial and fungal species richness as compared to organic. Organic and BD treatments were statistically indistinguishable from one another, and the additional input of BD preparations did not affect the fungal composition or richness as compared to the organic treatment. Fungal communities were also quantified in six conventional and six BD vineyards by Morrison-Whittle et al. (2017). By analyzing samples from several different vineyard "habitats" (i.e., bark, fruit, and soil) with metagenomic techniques, they found significantly higher species richness in BD fruit and bark communities, but not in soil. However, in terms of types and abundance of fungal species, BD management had a significant effect on soil and fruit. In terms of yields, an average yield reduction was also found in BD vineyard production systems as compared to in integrated systems, which amounted to -34% (Doring et al. 2015). This is probably due to plant health and disease incidence. Indeed, in this study, disease frequency of Botrytis was significantly increased in the BD treatment as compared to the integrated treatment where botryticides were applied. Furthermore, in a 3-year field trial in Italy, grape yields were found to not differ when comparing organic and BD treatments (Botelho et al. 2016), probably due to similar disease incidence levels. Botelho et al. (2016) also assessed physiological responses of grapevines to BD management and provided evidence of a strong stimulation effect of natural defence compounds in grape plants grown with BD preparations 500, 500 K, fladen, and 501. They found that BD management led to an increase in leaf enzymatic activities of chitinase and beta-1.3-glucanase as compared with organic management. Chitinase and glicanase activities are typically correlated with plant biotic and abiotic stresses and associated with induced plant resistance. Finally, they also found that the application of BD preparations reduced stomatal conductance and leaf water potential which indicated a higher water use efficiency (Chaves et al. 2010) in biodynamically managed vineyards. This is in line with Doring et al. (2015), who asserted that organic and BD treatments show significantly lower assimilation rates, transpiration rates, and stomatal conductance as compared to the integrated treatment. A reduction in stomatal conductance was then associated with enhanced tolerance of vine plants toward biotic (Zeng et al. 2010) and abiotic stresses (Salazar-Parra et al. 2012). In addition to the studies conducted on vineyards and arable cropping systems, our literature review found that a single article related to BD olive production in Tunisia (Blibech et al. 2012). Blibech et al. (2012) detected a high number of Bacillus species in olive groves managed with BD and organic methods.

After Choudhary and Johri (2009), these authors then supposed that an environment rich in organic substrates and micro-niches could support a complex of microbial species, in turn promoting the proliferation of Bacillus. Given the entomopathogenic role of Bacillus for several insects responsible for olive tree pests, they argued that BD and organic practices promote the bio-control of olive pests. This is the first study that showed the occurrence of Bacillus larvicidal strains in a BD olive tree farm that could be used in biological control programs. In addition to studies related to different production systems, we found studies dealing with single BD practices. Faust et al. (2017) found that, in a long-term field trial in Germany, the application of BD preparations did not give rise to any positive effects additional to those of composted farmyard manure fertilization. This is in line with Reeve et al. (2010), who report no differences in terms of pH, mineral elements, C/N ratio, NO3-N, and NH4+-N between a BD compost and an untreated compost. However, in a later study, Reeve et al. (2011) stated that, under changing circumstances, both the Pfeiffer field spray and other BD preparations were found to be moderately effective in raising soil pH. In terms of microbial activity, conflicting results are reported by Reeve et al. (2010) and Reeve et al. (2011). In the first study, they reported the occasional superiority of BD compost to untreated compost, but in the latter, no effect of BD compost was found. In addition, Reeve et al. (2011) found no effect on forage yield between fields treated with BD compost and with untreated compost but reported the occasional superiority of the impact of BD compost on wheat seedling height; results showed that a 1% extract of BD compost grew 7% taller wheat seedlings than a 1% extract of untreated compost did (Reeve et al. 2010). According to our selection of articles, there are only two surveys based on the manner of action of BD preparations. Giannattasio et al. (2013) performed a microbiological characterization of preparation 500 and identified some of its biological actions. They found that it is rich in enzymatic-specific activities and exhibits a positive auxin-like activity on plants but had no quorum sensing-detectable signal and no rhizobial nod gene-inducing properties. Moreover, they found that preparation 500 is relatively low in leucine aminopeptidase activity (an enzyme involved in nitrogen cycling), but enzymatic analyses indicated a bio-active potential in the fertility and nutrient cycling contexts. Another study aimed at characterizing the composition of BD preparations is that of Botelho et al. (2016), in which the concentrations of isopenthyl adenine, indole-3-acetic acid, and abscisic acid were below the detection limits. Moreover, the extremely low amount of plant regulators supplied by the BD preparations suggests that the hormonal mode of action proposed by Stearn (1976) is unlikely. This is in contrast with Giannattasio et al. (2013) who found that the indol-3-acetic acid activity and microbial degradation products qualify preparation 500 for possible use as soil bio-stimulants.

5.4.2 Results of the Literature Survey on Sustainability of the Biodynamic Method

There were 15 articles related to topic of the “sustainability of the BD method” (26) belonging to the first quartile of the corresponding WoS category and with IF > 2. The selection of most informative publications on the sustainability of the BD method is shown in Table 3 together with the geographical location of the trials, trial description, duration of the single experiments, and assessment method for measuring BD sustainability. Moreover, we classified sustainability based on the United Nations Millennium Declaration (2000) in which three domains of sustainability were distinguished: environmental, economic, and social sustainability. Most of the studies of the sustainability of the BD method are included in the environmental domain (8 studies), with there being only four and three studies respectively on economic and social sustainability. Having so few scientific studies on these topics available prevents us from drawing generic conclusions, especially if we consider that the vast majority of the studies mentioned do not show comparisons between different cultivation methods under a range of different influencing factors, such as soil type, climate or year of production, as can be argued from Table 3, where locations with corresponding pedo-climatic conditions, as well as years of experiments are reported.

5.4.2.1 Environmental Sustainability Agri-food is one of the sectors that contributes most to environmental impact in terms of resource depletion, land degradation, gaseous emissions, and waste generation (Cellura et al. 2012). There are several methods for assessing the agricultural impact on the environment, but life cycle assessment (LCA) is the most commonly used method as regards BD agriculture and one of the most commonly used in general. With this method it is possible to assess the environmental burden caused by a product, a production process, or any activity for providing services (Curran 2008). A decrease of environmental burden due to production activities measured with LCA was observed for BD viticulture in North-West Spain (Villanueva-Rey et al. 2014) and apricot production in Southern Italy (Pergola et al. 2017). Pergola et al. (2017) compared two integrated systems and one greenhouse managed under BD agriculture in an apricot orchard long-term field trial. They reported that BD practices led to higher environmental impacts due to the specific cultivation techniques used in BD greenhouse production. However, excluding the plantation phase from the analysis, the BD system consumed less energy and showed a favorable energy balance. Indeed, considering only cultivation operations, the production of 1 kg of integrated apricots required from 2.60 to 3.00 MJ kg^{-1} of energy, while the production of BD apricots required 1.32 MJ kg^{-1}. A lower environmental burden for BD production systems was also found by Villanueva-Rey et al. (2014) due to an 80% decrease in diesel input. This is in accordance with other studies (Alaphilippe et al. 2013; Bavec et al. 2012; Stavi and Lal 2013; Venkat 2012). In Bavec et al. (2012), a markedly reduced ecological footprint was found in organic and BD wheat and spelt production, mainly due to the absence of external production factors. When considering yields, the organic and BD systems had a reduced overall footprint per product unit and increased ecological efficiency of production. Soil carbon sequestration is a measure to prevent against CO_2 increase in the atmosphere and slow global warming (Janzen 2004; Page et al. 2011). Pergola et al. (2017) confirmed that, due to the soil management techniques used, the BD system fixed about 45% of the total CO_2 produced in the production cycle, with specific reference to soil. This is in line with Fließbach et al. (2007, Table 2) who found in the DOK trial that the soil organic carbon of the BD system was maintained at the same level for over 21 years and showed a small gain. This result is confirmed also by Reganold et al. (1993) and Droogers and Bouma (1996) comparing conventional and BD systems in which soil organic matter was proven to be stable only in the BD farming systems. According to Mäder et al. (2002), the energy to produce an organic crop dry matter unit was 20 to 56% lower than in conventional (Table 2). Indeed, nutrient input, energy, and pesticide were reduced by 34%, 53%, and 97%, respectively, in the organic systems, whereas mean crop yield was only 20% lower, indicating more efficient production. In addition, Nemecek et al. (2011a and 2011b) concluded that the environmental impacts per unit area were minimized in organic and low-input farming. However, resources and inputs (nutrients, water, soil) use efficiency is also necessary to implement environmental sustainability in farms. Indeed, the reduction of fertilizer use cannot be pushed too far without risking poor crop performance, and a minimum level of nutrient supply must be maintained to ensure good eco-efficiency (Nemecek et al. 2011b). This was also confirmed by Mayer et al. (2015), who found that, disregarding parameters of long-term soil sustainability, the conventional farming system at half standard fertilization displayed the best performance in terms of yields, crop quality, and efficiency.

5.4.2.2 Economic and Social Sustainability The lower BD yields are compensated for by higher prices for BD commodities and by additional subsidies (Nemecek et al. 2011b). Consumers are willing to spend more to acquire BD products (Bernabéu et al. 2007; ICEX, 2010) but, as suggested by the Greentrade marketplace (2006), the increasing number of farms shifting to BD agriculture will eventually lead to a steady convergence between conventional and BD prices. In our review, there were only two articles focusing principally on economic sustainability and the economic profit derived from BD and conventional farming systems (Table 3). Forster et al. (2013) considered economic performance in a cotton-soybean-wheat crop rotation in India. They found that soybean gross margin was significantly higher for the BD system (+ 8%) as compared to conventional system, and the slightly lower productivity of BD soybean was counterbalanced by lower production costs. However, this was not confirmed for wheat and cotton because of their low crop yield. The second study included in our literature selection was published by Reganold et al. (1993) and compared 16 BD and conventional farms in New Zealand. They found that the BD farms were just as financially viable on a per hectare basis as the conventional farms. Besides results on the economic and environmental sustainability of the BD method, we also found interesting outcomes from a social perspective. Following sociologist Bruno Latour's circulatory model of scientific work (Latour 1999), Ingram (2007) argued in the Annals of the Association of American Geographers that forms of alternative agriculture such as BD agriculture based on the "Going Back to Nature" paradigm were and have been the result of a scientific process characterized by an ongoing exchange of knowledge between scientists and farmers. BD networks have continued to consider farmers, especially those rejecting mainstream agriculture, as their primary counterpart (Ingram 2007). This is also confirmed by McMahon (2005), who interviewed six BD farmers in Ireland. However, he also found that some BD farmers restrict communication with the rural community and do not want to communicate the spiritual aspects of their farming methods, building from this perspective boundaries between them and "the Others."

5.4.3 Result of the Literature Survey on Food quality of Biodynamic products

There are 11 articles within the "food quality" topic (28) belonging to the first quartile of the corresponding WoS category and with IF > 2, with the first published in 2006 by Zörb et al. The selection of most informative publications on the food quality of BD products is shown in Table 4 together with the geographical location of the trials, trial description, BD relevant products, year of product harvest, size of experimental plots or samples, and parameters to assess food quality. In Zörb et al. (2006), a metabolite profiling of wheat (Triticum aestivum L.) grains was analyzed based on a total of 52 compounds. Only eight showed significant differences between organic and conventional systems, and no differences were found between organic and BD systems. Furthermore, Mayer et al. (2015) found that the conventional farming system at half standard fertilization had higher crude protein than organic and BD systems with standard fertilization and that doubling organic fertilization in organic and BD systems did not allow for improving grain baking quality. No differences between organic and BD systems were reported in terms of protein fractions, unextractable polymeric protein, gliadin, and dry gluten contents. In another field trial comparison, Turinek et al. (2016) investigated the composition of rapeseed (*Brassica napus L.*) seeds and found that BD and organic production systems positively influenced oleic fatty acid and oil content as compared to an integrated system. Conversely, the integrated system produced seeds with higher protein and water contents, as well as higher contents of linolenic, gadoleic, and hexadecadienoic fatty acids, due to mineral fertilizer application. Other studies comparing different management systems including BD farming were conducted on horticultural crops to study chemical composition and corresponding food quality. In an experiment conducted in Italy, the antiradical activity of chicory (*Cichorium intybus L.*) proved to be higher under BD than under conventional systems (Heimler et al. 2009). Such findings concerning antiradical activity were not confirmed by a following study carried out on Batavia lettuce (*Lactuca sativa L.*) in which, however, a higher amount of polyphenols was found under BD management (Heimler et al. 2012). Significantly, higher amounts of flavonoids and hydroxycinnamic acids in BD lettuce were detected as well, which was not the case for chicory. This last aspect could indicate an effect of BD practice on secondary metabolites in lettuce. In the abovementioned studies, the response of different crops to BD, organic and conventional management is not univocal and probably derives from several causes, including genetic characters and pedoclimatic conditions. Despite this, other studies report univocal outcomes in favor of BD agriculture, e.g., Bavec et al. (2010), who analyzed the chemical composition of red beet (*Beta vulgaris L.*) in a long-term field trial. They found that samples from BD plots had significantly higher total phenolic content, antioxidant activity, and malic acid content than samples from conventional plots, whereas total sugar content did not differ between production systems. In terms of number of studies, wine is the most common product to feature in BD food quality literature. Morrison-Whittle et al. (2017) evaluated the concentrations of volatile thiols important for aroma and quality in wines and found that there was no difference between BD and conventional wines. This was in line with Döring et al. (2015), who assessed grape quality comparing three farming systems (integrated, organic, and BD vineyards) and found that fruit quality in terms of total soluble solids, total acidity, and pH during ripening was not affected by the management system. However, BD treatment showed a significantly higher content of primary amino acids in healthy berries during maturation compared to the integrated treatment. Many other studies have argued that organic and BD viticulture have little influence on grape composition (Danner 1985; Hofmann 1991; Kauer 1994; Linder et al. 2006; Reeve et al. 2005). However, there is a trend for organic and BD juices to present higher contents of bio-active compounds as compared to conventional counterparts (Granato et al. 2016), and it is possible to differentiate organic/biodynamic and conventional purple grape juice through measurement of volatile organic compounds by proton transfer reaction mass spectrometry (Granato et al. 2015). Nevertheless, these and other studies found that BD and organic juices have very similar quality traits (Granato et al. 2015, 2016; Reeve et al. 2005), which is in line with the findings of Parpinello et al. (2015) who reported that the chemical and sensory properties of organic and BD wines do not differ. In terms of types and abundance of communities of fungal species in juice, Morrison-Whittle et al. (2017) found no differences between management systems. However, Mezzasalma et al. (2017) stated that natural berry microbiome could be influenced by farming management and pointed out that biodynamics had a consistent effect on the bacterial communities of berries and corresponding must. Animal-derived food is another important topic for understanding how the cultivation method can influence the quality of food. Capuano et al. (2014b) carried out an analysis of milk fatty acid profiles with cows from conventional, organic, and BD farms and found that organic/biodynamic milk differed from conventional milk. This was confirmed in a second part of their study (Capuano et al. 2014a), which analyzed the bovine milk by Fourier-transform infrared (FTIR) spectroscopy.

5.5 Discussion and Conclusion

5.5.1 Discussion of the Biodynamic Method

The aim of this review was to critically review the international scientific literature on BD agriculture as published in highly ranked journals, as well as to detect any lack of knowledge on relevant issues in agriculture. The results of the literature review showed that the BD method enhances soil quality and biodiversity, while no conclusion can be drawn regarding the socio-economic sustainability and food quality of BD products; further efforts needing to be made to implement knowledge of these aspects. Despite its being impossible to carry out a meta-analysis due to the small amount of data available and the vast range of differing parameters considered in the literature, some conclusive, semi-quantitative considerations can be drawn. To this end, we carried out a pairwise comparison exercise based on the results of BD, organic, and conventional agriculture regarding a vast range of parameters as published in highly ranked journals (IF > 2 and belonging to the first quartile of WoS corresponding categories). The results of pairwise comparison are shown in Table 5. The pairwise comparisons regarding the impact of agricultural practices showed that from a total of 74 observations comparing differences between BD and organic farming, 22 observed better performance from BD agriculture, 37 found equal performance, and 15 found better performance from organic agriculture. The comparison of BD and conventional farming showed that 44 observations found BD agriculture performed better, 12 found they performed equally well, and 14 found conventional agriculture performed better. Finally, comparisons between organic and conventional farming showed that 33 observations found organic agriculture performed better, 13 found equal performance, and 11 found conventional agriculture performed better. In terms of the sustainability of the BD method, the pairwise comparisons between BD and organic farming showed that one observation found in favor of BD agriculture, 24 found equal performance and two found in favor of organic agriculture, while the comparison between BD and conventional farming showed that 28 observations found BD performed better while seven found conventional agriculture did. Finally, the comparison between organic an conventional farming showed that 22 observations found organic performed better and four found conventional agriculture did. As regards the food quality of BD products, the pairwise comparisons between BD and organic farming showed that three observations found in favor of BD agriculture while 20 found equal performance. The comparison between BD and conventional farming showed that 13 observations found BD agriculture performed better, eight found no difference and seven found conventional agriculture performed better. Finally, the comparison between organic and conventional farming showed that four observations found organic agriculture performed better, 13 found no difference and four found better results from conventional agriculture.

It must be stressed that the majority of publications reporting organic/conventional comparisons in the overall literature do not examine BD agriculture; hence, the subset of articles cited in this manuscript does not rep-

Table 5: *Results of pairwise comparison between biodynamic/organic, biodynamic/conventional, and organic/conventional production systems grouped by three topics, i.e., impact of agricultural practices, sustainability, and food quality. + and - values were attributed based on counting of pairwise comparisons carried out in the literature for all the criteria reported in the first row of the table. The results of pairwise comparisons were standardized on a - 1/ + 1 scale, which was then transformed into five levels of performance ranging from - - , - , = , + and + +. It must be stressed that the majority of publications reporting ORG/CON comparisons in the overall literature do not encompass corresponding comparisons with BD agriculture; hence, the subset of comparisons upon which this table is based does not represent the entirety of ORG/CON comparisons in the literature*

	Impact of agricultural practices	Sustainability	Food quality
BD vs OR	=[c]	=[c]	=[c]
BD vs CO	+ BD[d]	+ +BD[e]	+ BD[d]
OR vs CO	+ OR[d]	+ + OR[e]	=[c]

[a] - -, Highly worse performance [b] - , Worse performance [c] = , Neutral result
[d] + , Better performance [e] + + , Highly better performance
BD, biodynamic agriculture; OR, organic agriculture; CO, conventional agriculture

resent the universe of organic/conventional comparisons in the literature, which greatly reduces the possibility of drawing generic conclusions in this matter. We have in any case reported the results of organic/conventional comparisons in BD agriculture publications as a reference for other comparisons within the set of publications analyzed in this article. BD agricultural practices promote overall agroecosystem biodiversity. BD farms usually maintain vegetative buffer strips, riparian corridors and hedgerows that provide shelter to pollinators and natural predators. Indeed, the Biodiversity Farm Programme imposed by Demeter Standards obliges 10% of total farm area to be dedicated to the care of biodiversity, which includes elements for the maintenance of rare or endangered plant and animal species, creating optimal conditions for insects, birds and in general all lifeforms, including soil microorganisms. One of the major challenges for all production methods is to provide enough nutrients to plants while promoting overall soil quality. To this aim, BD agriculture promotes close cycles using farm-produced animal and green manure instead of employing external organic fertilizer. Indeed, it is a general principle required by BD standards to include the animal element in any farming system to avoid imports of organic inputs and related nutrient imbalances. By contrast, in some cases such as those reported by Zikeli et al. (2017), high intensification of production in greenhouse systems backed by minimum compliance of BD standards led to strong imbalances in nutrient cycles. However, it should be noted that cases like those described by Zikeli et al. refer to unique production conditions in intensive horticultural systems subject to the exceptional derogation offered to smallholders. The combined effects of biodiversity management and nutrient cycling practices in BD agroecosystems seem to hold the potential to enhance soil microbiome. In our review, we found that overall microbial activity increased in BD farming systems as compared to conventional and organic agriculture (Mader et al. 2002; Fliebach et al. 2007). This was also confirmed by a recent meta-analysis by Christel et al. (2021), which found that 52% of microbial indicators were higher even in comparison with organic farming. In this article, BD farming appears as the farming system with the most favorable effect on soil ecological quality, followed by organic and, finally, conventional farming. This is in line with previous studies by and Droogers and Bouma (1996), who found that organic matter contents were higher in BD as compared to conventional fields. However, microbial activity and proliferation could be influenced not only by the farming system but also by differing supply of organic substrate, water availability, climate, and by the absence of pesticides. Overall, one of the most important issues to be addressed and promoted among farmers, whatever farming method they adopt, is that soil acts as a habitat for many living organisms that supply a vast range of ecosystem services including soil fertility, and that the maintenance of healthy soil is vital to fulfill the needs of those microbial populations. The third relevant aspect regarding the impact of agricultural practices focuses on the use of BD preparations Table 1. Turinek et al. (2009) reviewed the effects of BD preparations on yield, soil quality, and biodiversity and came to the conclusion that the natural science mechanistic principle backing BD preparations is still unclear and needs further investigation. Beyond a scarcity of information on BD preparations, our selection of articles reports conflicting results, which does not allow us to draw generic conclusions on related potential benefits. However, two studies not included in our selection suggest that preparation 500 could have the potential to stimulate plant growth (Spaccini et al. 2012) and that cow horns in which bovine fecal material is incubated for several months, could provide suitable substrates for a specific proteolytic decomposition process (Zanardo et al. 2020). Further studies are needed to test the activity of BD preparations under different conditions. The amount of selected articles on the sustainability of the BD method is notably low, which hinders the possibility of reaching robust conclusions. Most outcomes found in the literature on the sustainability of BD agriculture concern the environmental aspects, while socio-economic considerations are scarcely considered. Indeed, the results of the pairwise comparisons which focused exclusively on environmental sustainability showed that, from a total of 21 observations comparing the difference between BD and organic farming, one observation found in favor of BD agriculture, 19 found no difference and one found in favor of organic agriculture. The comparison between BD and conventional farming showed that 24 observations found BD to be better while 4 found for conventional agriculture. Finally, the comparison between organic and conventional farming showed that 19 observations found in favor of organic and two in favor of conventional agriculture. Hence, as regards environmental sustainability, there appears to be robust evidence in the literature of the fact that BD agriculture greatly outperforms conventional agriculture, while no difference has been detected as compared to the performance of organic agriculture. At the farm economics level, our review confirms that remuneration of BD farmers appears to be equal or even considerably more profitable on a per hectare basis than conventional farming. This was confirmed on a national scale by the 2019 Bioreport published by the Italian Ministry of Agriculture, which stated that the turnover per hectare of Italian BD farms was in general higher as compared to conventional farms (i.e., 13.300 versus 3.207 euro/ ha, Rete Rurale Nazionale 2019), and also by Penfold et al. (1995) who reported that BD system had the highest gross margins as compared to conventional, organic, and integrated systems. This might also be due to lower production costs and supply of wider range of goods and services producing income diversification in BD farms (Mansvelt et al. 1998). On the other hand, Aare et al. (2020) found that extra costs connected to diversification in BD farms do not generally pay off on standard food markets because of equal prices of organic and BD products, which leads BD farmers to export their products to countries like Germany and France where they can achieve 20% higher prices on average. Finally, the results of the review of literature on social sustainability regard only two publications and are thus wholly insufficient to allow any generic conclusions on BD agriculture. As regards the impact on food quality, BD agriculture performs slightly better than conventional while no difference was detected when comparisons between BD and organic were carried out. Though the food quality of BD products is at an early stage of development in the literature, some general remarks can be made concerning BD agriculture performances in relation to nutritional properties, which are the most frequently addressed topic in the scientific literature on the quality of food from BD agriculture. The outcomes of our review show BD products to be nutritionally richer than conventional counterparts. Other studies not included in our selection confirmed that nutritional properties, in particular the content of phenolic compounds, flavonoids, and antioxidant activity were significantly higher in strawberries, mangoes, and grapes from BD farming as compared to conventional (respectively, D'Evoli et al. 2010; Fonseca Maciel et al. 2011; Reeve et al. 2005). However, dietary health is not only a matter of the nutritional value of food but also the result of how the soil microbiome interacts with plants, animals, and humans. Indeed, the concept of One Health proposes that there is a connection between human, animal, and environmental health (Karesh et al. 2012; Wolf 2015). Van Bruggen et al. (2019) argued that the health conditions of all organisms in an ecosystem are interconnected through the cycling of subsets of microbial communities from the environment (in particular the soil) to plants, animals, and humans. The One Health approach combined with better performances of BD soils in terms of microbial indicators as previously reported (Christel et al. 2021) might therefore support the idea that BD products are healthier.

5.5.2 Need for a Systemic Approach

One frequent observation on the robustness of the results analyzed in this review of the literature regarding BD agriculture is that they can be greatly affected by production and site-specific conditions of relevant experiments. This aspect is common to all fields of research in agriculture but becomes, if possible, even more important when we investigate agroecological types of farming, including BD and organic agriculture. Systems theory holds that the behavior of any system in a hierarchy, e.g., the farm system, is not readily discoverable from a study of lower systems, e.g., cropping/livestock systems, and vice versa (Checkland 1981; Milsum 1972; Simon 1962; Whyte et al. 1969). The behavior of a system is instead a consequence of the combination of impacts of decisions taken at different levels in the hierarchy. Each level in the hierarchy could be related to any other, within and between levels (Conway 1987). As a reaction against the reductionist approach which emphasizes the simplification of the system, agroecological thinking resulted in the development of an "agroecosystem" view (Conway 1987; Marten 1988), which promotes the need for a holistic and systemic approach to agroecosystems analysis. Systems theory (Bertalanffy 1968; Morin 1993; Odum 1989; Prigogine 1980) is an analysis method which describes interactions between components of the system and aims for a better understanding of system complexity. Any application of the theory of scaling should take into consideration the complex interactions between biophysical, social, economic, and institutional factors to analyze and understand the relations that characterize farming systems (Marchetti et al. 2020; Wigboldus et al. 2016). However, as reported by Schiller et al. (2019), limited analysis of how technological, political, and financial factors interact has been performed, and the evaluation of agroecosystem factors is complicated by their high dependence on the environmental and social conditions in which they are applied (Marten 1988). Current methods of analysis do not sufficiently consider system complexity and are based on the premise of "find out what works in one place and do more of the same in another place" (Wigboldus et al. 2016). Agricultural systems such as BD agriculture require more research based on a systemic approach which considers interconnections between ecological, economic, social, and political variables. A system thinking perspective on BD agriculture, as well as for other forms of agriculture, has to be conceptualized, and may serve as a basis for future research. The best solutions for achieving a systemic approach for agroecological transitions might be found by integrating disciplines that explore the diversity and synergies of relationships between the various levels involved (Comeau et al. 2008; Ollivier et al. 2018; Wigboldus et al. 2016). This may require new expertise with the aim of facilitating collaborative processes (Brouwer et al. 2016; Hermans et al. 2013; Schut et al. 2011; Spruijt et al. 2014; Turnhout et al. 2013; Wigboldus et al. 2016; Wittmayer and Schäpke 2014). Moreover, as reported by Ollivier et al. (2018), beyond scientific disciplines, agroecological transition requires increasing knowledge through experiential and social learning processes within trans-disciplinary epistemological research, involving farmers in all stages to cultivate new sustainable cultural approaches (Marchetti et al. 2020). It is necessary to innovate across all agri-food systems through forms of participatory research, which implies the involvement of farmers and consumers, and re-establishing producer–consumer connections. While considering issues of experimental design, trials should minimize or eliminate confounding variables which can offer alternative explanations for the experimental results. For example, if BD and organic farmyard manure treatments are obtained from two different farms, differences could be caused not only by the biodynamic preparations but also by the different manure qualities (Heinze et al. 2010). Finally, as suggested by several authors (Bàrberi et al. 2010; Perry 1997), it is important that the experimental design includes large plots ensuring adequate replication in trials to avoid methodological spatial problems linked to heterogeneity of site-specific conditions. In conclusion, BD agriculture offers promising contributions for the future development of sustainable agricultural production and food systems, but the extent to which relevant results can be considered scientifically reliable depends on a systemic and participatory approach being applied when addressing real-world business challenges.

5.5.3 Concluding Remarks

Scientific research into BD agriculture seems still to be at too early a stage of development to allow for reasonable, generic conclusions about its performance as a production method. All the topics so far analyzed need further study in order to allow relevant conclusions about different pedo-climatic, production and even cultural conditions to be made. Nevertheless, some tentative conclusions can be drawn. The results of the literature review showed that the BD method enhances soil quality and biodiversity. Many of these results were generated in long-term trials where the temporal dynamics of soil indicators could be studied. Further efforts need to be made, however, to understand the socio-economic sustainability and food quality aspects of BD products. One particularly promising topic of research consists in the assessment of microbial activity and the potential that the microbiome has in BD farms to enhance soil fertility and human health following the One Health approach. If such results could be obtained in BD agriculture by improving biodiversity management and nutrient cycling through animal rearing in farms or simply by applying BD preparations, the topic could be included in the research agenda. Moreover, it is critical to take a systemic approach to investigating similar subjects. We can therefore conclude that BD agriculture could provide benefits to the environment and that more research and innovation activities should be undertaken in order to provide additional information to farmers, policy makers, and stakeholders about this type of organic agriculture.

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6 Soil Amendment Strategies Using Agroecological Practices in a Long-Term Experiment in Tuscany (Italy)

Margherita Santoni[1], Ottorino-Luca Pantani[1], Francesco Serafini[1], Lorenzo Ferretti[1], Carlo Viti[1], Matteo Daghio[1], Gaio Cesare Pacini[1]

[1] Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali (DAGRI) - University of Florence (Italy)

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6.1 Abstract

Organic farming systems in the Mediterranean area are often stockless. The stockless management eventually results in a scarcity of soil organic matter, which in turn is thought to be the main hurdle in coupling soil fertility with crop nutrition. Therefore, it seems necessary to investigate fertilization solutions that are able to reconnect crops and animal production, thus allowing the local unfolding of nutrient element cycles. This study is an attempt to carry out a systemic soil fertility assessment which includes a wide set of indicators regarding chemical, physical and biological soil properties to test different type of organic amendments such as pelleted manure, fresh manure and biodynamic compost. To date, the tested amendments have not influenced the tested indicators. Future developments entail more comprehensive analyses on these indicators. Moreover, an additional biological indicator, i.e. soil micro-arthropods, will be included in this research.

Keyword: soil health, soil quality, Mediterranean area, biodynamic agriculture, organic agriculture, amendment

6.2 Introduction

Organic farming systems in the Mediterranean area are often stockless (Canali et al., 2005), even if its basic principles are based on the functional interconnection between crops and animal productions. Stinner et al. (2008) found an increase in the number of European farmers operating in stockless organic systems. Obviously, the stockless management eventually results in a scarcity of soil organic matter, which in turn is thought to be the main hurdle in coupling soil fertility with crop nutrition (Berry et al., 2002; Cormack et al., 2003; Stinner et al., 2008). In organic systems, soil fertility is strictly required to maximize the resilience to climatic and environmental variations on the long term. The current trend is to concentrate livestock farms in limited areas, which results in two problems:

- excessive concentration of manure in nearby fields, which could result in nitrate contamination in water bodies
- limited or null availability of manure in other areas, mostly because of the excessive transport costs.

Organic farmers were thus obliged to close the elements' cycles outside their farm, acquiring organic materials produced elsewhere: this externalization is a phenomenon which has been described as *conventionalization of organic farming* (Darnhofer et al., 2009).

Organic farmers in the Mediterranean area maintain the fertility of their soils using organic amendments such as dried or pelleted manure, fresh manure, vermicompost, compost of food industry residues, etc. However, from a biological standpoint, biodynamic compost has been found to possess bio-active potential in the contexts of fertility and nutrient cycling (Giannattasio et al., 2013).

Biodynamic agriculture proposes an agroecological model which is based on a closed production system that includes livestock within the farm. This model focused on reducing energy consumption, achieving high levels of environmental efficiency, and economic profitability (Bioreport, 2018).

Based on the current long-forecaster energetic crisis, it seems necessary to investigate amendment solutions that are able to reconnect crops and animal production, thus allowing the local unfolding of nutrient element cycles. This study is an attempt to carry out a systemic soil fertility assessment which includes a wide set of indicators regarding chemical, physical and biological soil properties to test different type of organic amendments such as pelleted manure, fresh manure and biodynamic compost. The hypothesis at issue is which of these organic soil amendments can enhance soil fertility. Since that soil fertility is featured with long-term dynamics, we therefore carried out our analyses at the Montepaldi Long Term Experiment (MoLTE, San Casciano Valdipesa, Florence, Tuscany), which is the longest experiment on organic farming of the entire Mediterranean area.

6.3 Materials and Methods

6.3.1 Description of the Experimental Site

The experimental site is located in the “Montepaldi Long Term Experiment” (MoLTE), location Montepaldi, San Casciano Val di Pesa, Italy, Long. 11°09’08’’ E, Lat. 43°40’16’’ N, 90 m a.s.l. (Figure 1). The MoLTE has been active since 1991 and is unique in Italy and over all the Mediterranean area for its duration and quantity of data collected[4].

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 1.

The MoLTE is divided into three stockless arable systems:

(i) an organic system, named Old Organic (*OldOrg*), certified as organic agriculture since 1992 (EC reg. 2092/91 and following regulations)

(ii) an integrated one (EC regulations 2078/92) until 2001, which was then converted to organic (New Organic - *NewOrg*).

(iii) a conventional/high-input system, where xenobiotics and synthetic fertilizers have been routinely applied since 1992.

Since this study is focused on organic farming, only (i) and (ii) were considered. The typical fertilization intensity found in ordinary organic farms in the region has been used in MoLTE, which consists in using organic fertilizers, amendments and green manure. From 2020 to 2023, a three-year rotation consisting of spelt (*Triticum dicoccum L.*), ancient common wheat (*Triticum aestivum L.*) and alfalfa (*Medicago sativa L.*) was adopted in both systems. In Table 2 additional agronomic details are shown.

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

Table 1: *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)	8.30	8.3
C.E.C. (meq. 100 g^{-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 2: *Agronomic details from 2020 to 2023 at MoLTE.*

	2020-2021		2021-2022		2022-2023	
Management	Old Organic	New Organic	Old Organic	New Organic	Old Organic	New Organic
Previous crop	*Triticum dicoccum L.*	*Triticum dicoccum L.*	*Triticum aestivum L. var. Gentil-rosso+Andriolo*	*Triticum aestivum L. var. Gentil-rosso+Andriolo*	*Medicago sativa L.* var. Maraviglia	*Medicago sativa L.* var. Maraviglia
Actual crop	*Triticum aestivum L. var. Gentil-rosso+Andriolo*	*Triticum aestivum L. var. Gentil-rosso+Andriolo*	*Medicago sativa L.* var. Maraviglia	*Medicago sativa L.* var. Maraviglia	*Medicago sativa L.* var. Maraviglia	*Medicago sativa L.* var. Maraviglia
Plant density	160 kg ha^{-1}	160 kg ha^{-1}	40 kg ha^{-1}	40 kg ha^{-1}	-	-
Plowing	Sept/13/2020	Sept/13/2020	Sept/11/2021	Sept/11/2021	-	-
Disk harrowing	-	-	Apr/19/2022	Apr/19/2022	-	-
[a]Fertilization	Sept/12/2020	Sept/12/2020	Sept/10/2021	Sept/10/2021	-	-
[b]Distribution of biodynamic preparations 500	Apr/20/2021 May/5/2021	Apr/20/2021 May/5/2021	Apr/7/2022	Apr/7/2022	Mar/13/2023	Mar/13/2023
Sowing	Nov/25/2020	Nov/25/2020	Apr/20/2022	Apr/20/2022	-	-
Weed harrowing	Mar/4/2021	Mar/4/2021	-	-	-	-
Harvest	Jul/23/2021	Jul/23/2021	-	-	Jun/1/2023 Jul/27/2023	Jun/1/2023 Jul/27/2023

[a] Based on the experimental design (sec. 3.2). [b] Only in plots treated with biodynamic manure, *BdMa* (sec. 3.2).

Figure 1: *Location of the Montepaldi Long Term Experiment (MoLTE).*

6.3.2 Description of the Experimental Set-up

A randomized complete block design with two factors was used for this experiment:

- MANagement, with two levels
  - *OldOrg*
  - *NewOrg*
- TReaTment, with five levels
  - *NoNe*, control, i.e. without amendment.
  - *PeMa*, pelleted cow manure at 1.5 ton ha^{-1}.
  - *OrMa*, fresh organic cow manure from an organic certified farm at 30 ton ha^{-1}.
  - *BaMa*, fresh organic cow manure from an organic certified farm, added with biodynamic preparations and then composted at MoLTE for six months at 8 ton ha^{-1}.
  - *BdMa*, cow compost from a biodynamic certified farm composted for six months at 8 ton ha^{-1}.

The four types of organic amendments were applied in the fields twice, in September 2020 and September 2021 (Table 2). The fresh organic cow manure of *OrMa* and *BaMa* were obtained from the same organic certified farm (Agri-Ambiente Mugello, Florence). The doses per hectare are not constant among the levels of TRT since they represents the ordinary amounts used in organic and biodynamic farms. In Table 3 are listed the biodynamic preparations together with their main ingredient, mode of use and predicted influence (Turinek et al., 2009).

One field per system — 0.4725 ha each — was divided into 3 horizontal strips (REP, see Figure 2), separated by a corridor. In each strip, 5 plots were randomly assigned to one level of fertilization (TRT). Within each plot, 20 polygons with equivalent area were drawn (Walvoort et al., 2023) and a couple of xy coordinates were randomly generated within each polygon (Figure 3). Finally, 4 xy coordinates were randomly selected among the 20 couples (SUB-REP) and there the indicators were always sampled, once or repeatedly along TIME.

From a previous study conducted at MoLTE (Pantani et al., 2022) was observed that earthworms, used as a biological indicator in this study, demonstrated susceptibility to operators' trampling. Therefore, to mitigate the influence of this factor, each SUB-REP of earthworm sampling was randomize following a chronological order, as exemplified in Figure 4.

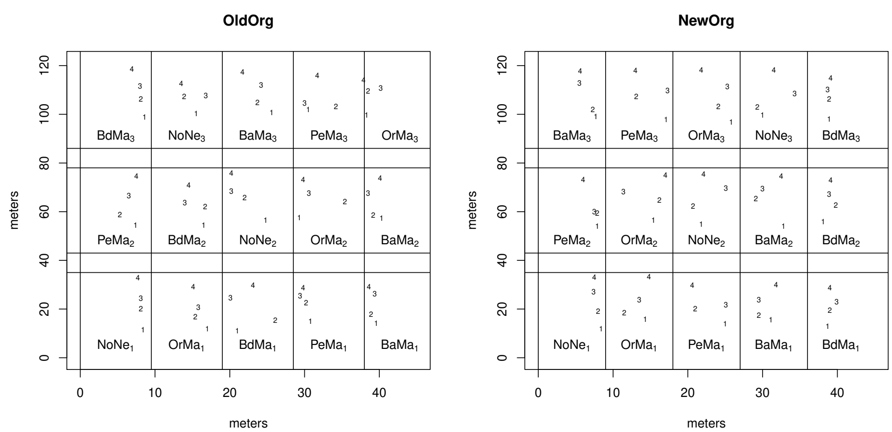


Figure 2: *Field experimental design at MoLTE. The abbreviations NoNe, PeMa, OrMa, BaMa and BdMa, i.e TRTs, indicate no amendment, pelleted cow manure, fresh organic cow manure from an organic certified farm, fresh organic cow manure from an organic certified farm added with biodynamic preparations and then composted and cow compost from a biodynamic certified farm, respectively. Numbers subscripted 1, 2, 3 indicate the replicate (REP). The abbreviations OldOrg and NewOrg indicate Old Organic and New Organic managed fields (MAN), which is composed by 15 plots. Each plot is 9 mt wide and 35 mt long. In each plot 4 sampling sites (SUB-REP) were fixed for the entire duration of the experiment.*

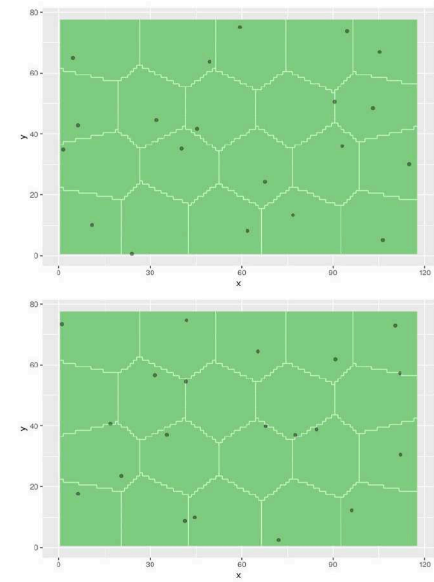


Figure 3: *The spatial distribution of sampling site. Each perimeter encloses a sampling area of the same size. The xy coordinates of the sampling points (SUB-REP) were randomly assigned within each area.*

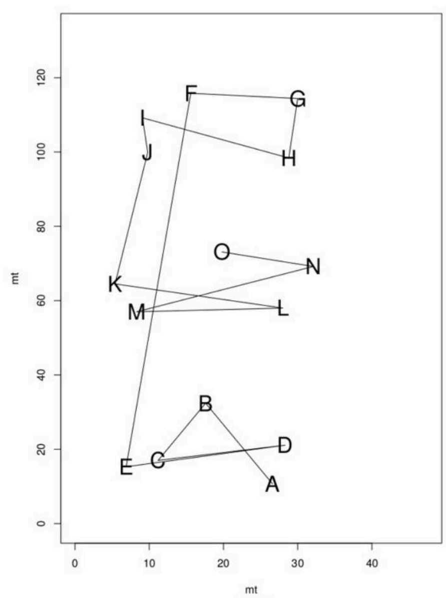


Figure 4: *Earthworm sampling was randomized within each REP with a chronological order. Capital letters indicate the sampling order, progressing from A to E, then from F to J, and finally from K to O.*

Table 3: *Details of biodynamic preparations used in composting fresh organic cow manure (BaMa)*

Number of the preparation	Main ingredient	Use	Mentioned in connection with
500	Cow manure	Field spray	Soil biological activity
501	Silica	Field spray	Plant resilience
502	Yarrow flowers (*Achillea millefolium L.*)	Compost preparation / inoculant	K and S processes
503	Chamomile flowers (*Matricaria recutita L.*)	Compost preparation / inoculant	Ca and K processes
504	Stinging nettle shoots (*Urtica dioica L.*)	Compost preparation / inoculant	N management
505	Oak bark (*Quercus robur L.*)	Compost preparation / inoculant	Ca processes
506	Dandelion flowers (*Taraxacum officinale Web.*)	Compost preparation / inoculant	Si management
507	Valerian extract (*Valeriana officinalis L.*)	Field spray, compost preparation / inoculant	P and warmth processes

__Table 4:__ Sampling date for each indicator during the experiment.

	Sampling date
Chemical indicators[a]	
Organic carbon	Sept/11/2020; Sept/10/2021; Sept/23/2022
Total N	Sept/11/2020; Sept/10/2021; Sept/23/2022
Total and available P_2O_5	Sept/11/2020; Sept/10/2021; Sept/23/2022
Physical indicators	
Aggregate stability[b]	Sept/11/2020; Sept/23/2022
Soil penetration resistance[c]	Mar/17/2021; Mar/22/2021; Apr/7/2022; Oct/7/2022; Apr/5/2023
Biological indicators	
Earthworm	Mar/3/2021; Apr/19/2021; Apr/6/2022; Apr/16/2022; Mar/16/2023
Soil microbial communities[a]	Sept/23/2022
Weeds	Apr/26/2021; Sept/29/2022; Apr/14/2023
Ancient common wheat yield	Jun/30/2021
Alfalfa yield	Apr/28/2023

[a] Only 60 out of 120 samples were analysed [b] Only 30 out of 120 samples were analysed
[c] 4 samples for each SUB-REP were collected

6.3.3 Chemical and Physical Indicators

6.3.3.1 Organic Carbon, Organic N and Total and Available P_2O_5
The soils were sampled in September 2020, 2021 and 2022 (Table 4). For each sample, the following chemical indicators were measured: organic carbon and organic N content by flash combustion (CIT), and total and available P_2O_5 (Olsen et al., 1954).

6.3.3.2 Aggregate Stability Soil aggregate stability in water was performed on air dried samples. In order to obtain insight into slaking — the aggregate breakdown due to internal stresses caused by rapid water uptake that compresses air — 300 mg aliquots of calibrated aggregates (0.5–1 mm) both dry and pre-wetted by gently spraying deionised water were immersed in distilled water circulating in a wet sample dispersion unit of a laser granulometer analyzer (Malvern Mastersizer 2000). The fragment/particle size distribution of suspended material was recorded after each minute for 12 min for about 24 minute. The median diameter (equivalent diameter d_{50}) of the particle-size distribution, interpolated with a logarithmic function, was assumed as an estimate of soil aggregates stability. The entire dataset (changes in particle size distribution over time) was also analyzed compositionally as described in the data analysis section. A total of 30 dry + 30 wet samples (2 MAN * 5 TRT * 3 REP * 2 SUB-REP) both at the beginning and the end of experiment (2020 and 2022) were analyzed (Table 4).

6.3.3.3 Soil Penetration Resistance The penetrometry measurement (0–50 cm) was performed with an hand penetrometer EiJkelkamp (Figure 5). For each sampling session, four samples were collected for each SUB-REP (480 measurements - see Table 4).

Figure 5: *Soil penetration resistance was performed with an hand penetrometer EiJkelkamp*

6.3.4 Biological Indicators

6.3.4.1 Earthworms Earthworms' abundance was estimated by the VESS method (Ball et al., 2007), which consists in the extraction and exploration of a soil cubic block (30 cm side). During the exploration, the soil block was destroyed and the numbers of earthworms and their age (baby, young and adult) were recorded.

As established by genome sequencing (Pantani et al., 2022), earthworms population was entirely composed of anecic ecotype (Paoletti et al., 2013), i.e. *Hormogaster samnitica* species. In Table 4, earthworms sampling dates were reported. A total of 120 soil cubes for each sampling session (2 MAN * 5 TRT * 3 REP * 4 SUB-REP) were collected.

6.3.4.2 Soil Microbial Communities Soil microbial communities were analyzed at the end of the experiment (2022) after two organic amendment distributions (Table 2). Indeed, it would be redundant to assess the microbial communities at the beginning of the experiment if no discernible outcomes were to be observed following the two distributions. A total of 60 soil samples collected in September 2022 (2 MAN * 5 TRT * 3 REP * 2 SUB-REP) were analysed (Table 4). Soil samples were thawed in ice and the total DNA was extracted using the FastDNA™ SPIN Kit for Soil (MP Biomedicals) following the manufacturer's instructions. The fungal ITS2 was amplified using the primers ITS3_KYO2 (5'-GATGAAGAACGYAGYRAA-3') and ITS4r (5'-TCCTCCGCTTATTGATATGC-3') (Toju et al., 2012; White et al., 1990). Amplicons preparation and sequencing were performed at BMR Genomics Srl (Padova, Italy) by MiSeq Illumina (Illumina, Inc., San Diego, CA, USA) using a 300 bp 2 paired-end protocol. Bioinformatic elaborations were performed as follows: primers were removed using cutadapt v3.5 (Martin, 2011). Further bioinformatics elaboration was performed using usearch v11 (Edgar, 2016). Forward and reverse reads were merged, and a quality filter was applied (maximum expected error threshold = 1.0). The reads were dereplicated and error-correction of amplicon reads was performed using UNOISE algorithm (Edgar & Flyvbjerg, 2015) with default parameters to generate the zero-radius Operational Taxonomic Units (zOTUs) and chimera were removed. The reads were mapped against the zOTUs with default parameters. Taxonomic assignment for each zOTU was performed against the UNITE database (Kõljalg et al., 2005). Both Chao1 index and the Shannon diversity index were calculated to estimate the alpha-diversity. The alpha diversity was estimated on a randomly rarefied dataset (8,263 sequences).

6.3.4.3 Abundance and Biomass of Weeds Weeds assessment was based on sampling field portions of 0.25 m^2 following the throwing of a square metal sampling frame. The frame was thrown randomly for each SUB-REP and all weeds found within the frame perimeter were removed. The weeds were then grouped by species and the number of individuals for each species and the dry weight (drying at 60°C) per species were recorded, i.e. weeds abundance and biomass, respectively.

6.3.4.4 Yield For each SUB-REP, the sampling procedure described for weeds, was used for common wheat and alfalfa plants, the two crops cultivated from 2020 to 2023. After drying grains and fodder at 60°C, dry matter yield (ton ha^{-1}) was then estimated for common wheat and alfalfa, respectively.

6.3.5 Statistical Analysis and Data Treatment

The analytical process was as follows.

(i) to provide an overall summary of the data, the indicators were analyzed and ANOVA followed by a HSD Tukey test were performed, except for number of earthworms, since this data showed deviation from normality;

(ii) earthworm abundance were treated as counts and analysed with Generalized Linear Models (GLM), with a Binomial distribution and a log link function;

(iii) data from aggregate stability were considered as compositional, sensu Aitchison (1986);

(iv) soil microbial communities were analysed by a non-metric multidimensional scaling (NMDS) and a permutational multivariate analysis of variance (PERMANOVA) based on Hellinger transformed zOTUs abundance data. Both the NMDS and the PERMANOVA were performed on the weighted Bray-Curtis distances. The taxa with a different relative abundance between the conditions were identified by a Kruskal-Wallis test and multiple comparison was performed by a Dunn test (p-values were corrected using the Benjamini-Hochberg adjustment);

(v) for each data class in i) and ii), comparison of marginal models was used in order to find the simplest model — the one with the least number of significant descriptors — capable of describing the data variability. For data class in i), ANOVA was performed on the final model for each indicator and analysis of residuals did not show substantial deviation from normality.

The statistical analyses were performed using R statistical software, version 4.3.2 (R Core Team, 2023) and some of its libraries (Callahan et al., 2016; Oksanen et al., 2022; Sarkar, 2008; Venables & Ripley, 2002).

Linear and generalized linear models were built by lm() and glm() functions. The reference treatment (REF-TRT) for TRT variable has been set as *OldOrg-NoNe*. The dropterm() and stepAIC() functions (Venables & Ripley, 2002) were used to explore the model space for lm and glm R classes, while for acomp classes the exploration of model space was performed manually, following the indications of Boogaart & Tolosana-Delgado (2013). For NMDS and PERMANOVA, data were performed using the metaMDS and the adonis2 functions, respectively.

Data obtained in the field and in the laboratory were processed according to the reproducible research protocols[5]. A free and open source distributed version control system was used to keep track of the changes in code writing, data analyses and so on.

[5] https://git-scm.com/

6.4 Results

6.4.1 Chemical and Physical Indicators

6.4.1.1 Organic Carbon The results for organic carbon are presented in Figure 6, Figure 7 and Table 5. The organic carbon content showed a parabolic trend (TIME not significant; TIMEˆ2: significant - Table 5) over the years. Moreover, a significant difference between the two systems (MAN) and for the MAN:TRT interaction were observed. In particular, organic carbon showed a lower content for *OldOrg-PeMa* and *OldOrg-BaMa* compared to other TRTs, while a higher value was recorded for *NewOrg-BaMa* (Figure 7). Nevertheless, organic carbon differs for all the TRTs under analysis even at the beginning of the experiment: it increases and decreases with the same shared curvature for all the TRTs, returning to the initial values after the amendment distribution (Figure 7). This trend was also observed for the REF-TRT, where no fertilizer was applied.

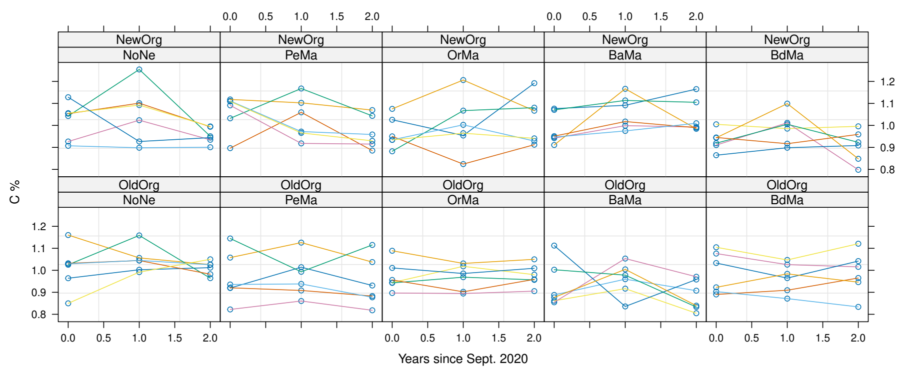


Figure 6: *Organic carbon during the experiment. The abbreviations NoNe, PeMa, OrMa, BaMa and BdMa, i.e TRTs, indicate no amendment, pelleted cow manure, fresh organic cow manure from an organic certified farm, fresh organic cow manure from an organic certified farm added with biodynamic preparations and then composted and cow compost from a biodynamic certified farm, respectively. The abbreviations OldOrg and NewOrg indicate Old Organic and New Organic managed fields (MAN). REP indicates the replicates.*

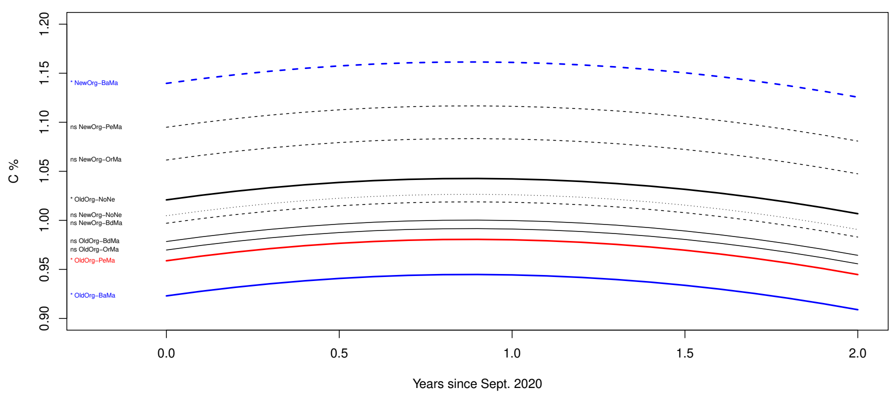


Figure 7: *Organic carbon content over the years (from September 2020 to September 2022) as influenced by treatment, TRT (NoNe-no amendment, PeMa-pelleted manure, OrMa-organic manure, BaMa-organic manure + biodynamic preparation, BdMa-biodynamic manure). The abbreviations OldOrg and NewOrg indicate Old Organic and New Organic managed fields (MAN). Solid black line represents the REF-TRT, i.e. Oldorg-NoNe. Dotted black lines represent the TRTs not significantly different (p <= 0.05) from REF-TRT. Solid and dotted colored lines represent the TRTs significantly different (p <= 0.05) from the REF-TRT.*

Table 5: *Analysis of Variance (ANOVA) for organic carbon during the experiment.*

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Time	1	0.006	0.0059	0.86	0.36
Time^2	1	0.032	0.03	4.67	0.03
MAN	1	0.030	0.0300	4.34	0.04
TRT	4	0.057	0.0143	2.07	0.09
MAN:TRT	4	0.117	0.0293	4.24	$< 10^3$

6.4.1.2 Organic N The results for organic nitrogen are shown in Figure 8 and Figure 9. The trend in organic nitrogen mirrors that of organic carbon: it increases and decreases over the years, returning to the initial values after the amendment distribution (Figure 8). However, no significant differences were observed for all the experimental factors considered (Figure 9). This could be attributed to the general tenfold lower concentration of nitrogen content compared to organic carbon.

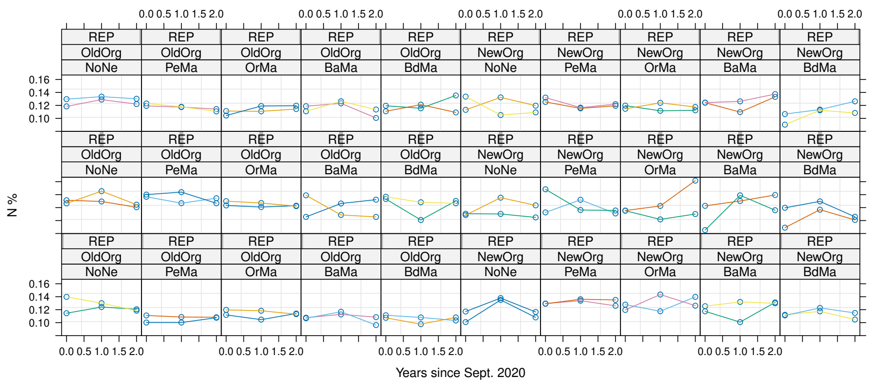


Figure 8: *Organic N content during the experiment. The abbreviations NoNe, PeMa, OrMa, BaMa and BdMa, i.e TRTs, indicate no amendment, pelleted cow manure, fresh organic cow manure from an organic certified farm, fresh organic cow manure from an organic certified farm added with biodynamic preparations and then composted and cow compost from a biodynamic certified farm, respectively. The abbreviations OldOrg and NewOrg indicate Old Organic and New Organic managed fields (MAN). REP indicates the replicates.*

6.4.1.3 Total and Available P_2O_5 The results of total P_2O_5 are presented in Figure 10, while available P_2O_5 is shown in Figure 11 and Figure 12.

The total P_2O_5 showed a significant difference between MAN, with *NewOrg* being higher than *OldOrg* by approximately 67 ppm (Figure 10). However, no significant differences were observed among TRTs.

The available P_2O_5 significantly increased after the first amendment in 2021, then decreasing again in 2022 (Figure 11). This trend was validated using a mixed-effects model, revealing that *OrMa* and *PeMa* showed a higher available P_2O_5 content in 2021, followed by a subsequent decrease in 2022 (Figure 12). Therefore, a similar pattern of organic carbon and nitrogen was observed. No differences were observed among TRTs.

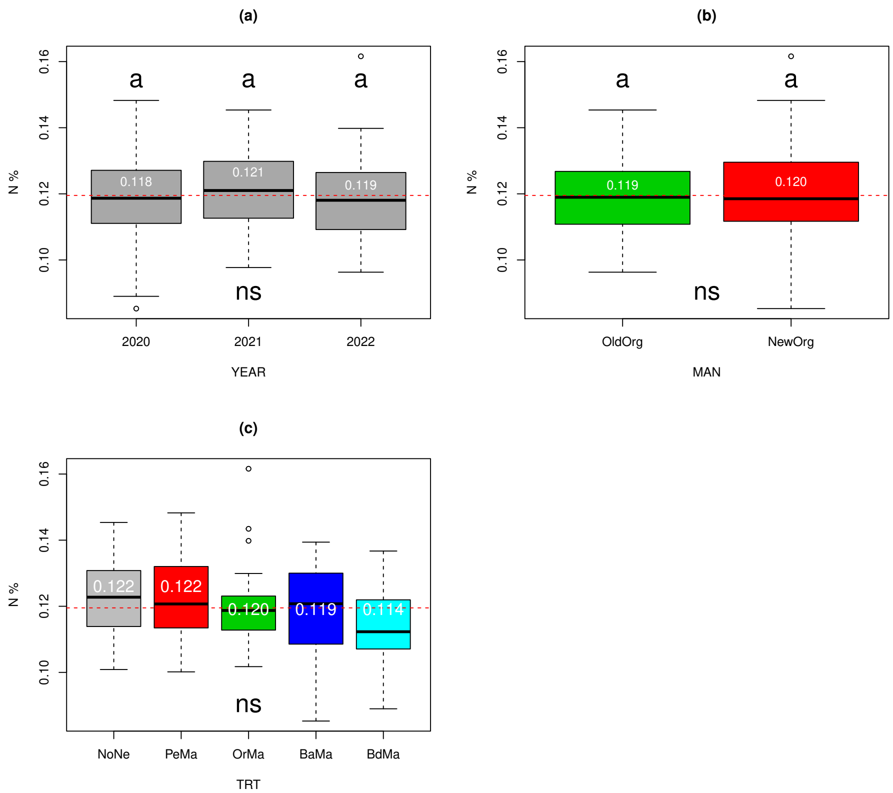


Figure 9: *Organic N content during the experiment. No difference were found among TIME (a), MAN (b) and TRTs (c). The abbreviations NoNe, PeMa, OrMa, BaMa and BdMa, i.e TRTs, indicate no amendment, pelleted cow manure, fresh organic cow manure from an organic certified farm, fresh organic cow manure from an organic certified farm added with biodynamic preparations and then composted and cow compost from a biodynamic certified farm, respectively. The abbreviations OldOrg and NewOrg indicate Old Organic and New Organic managed fields (MAN).*

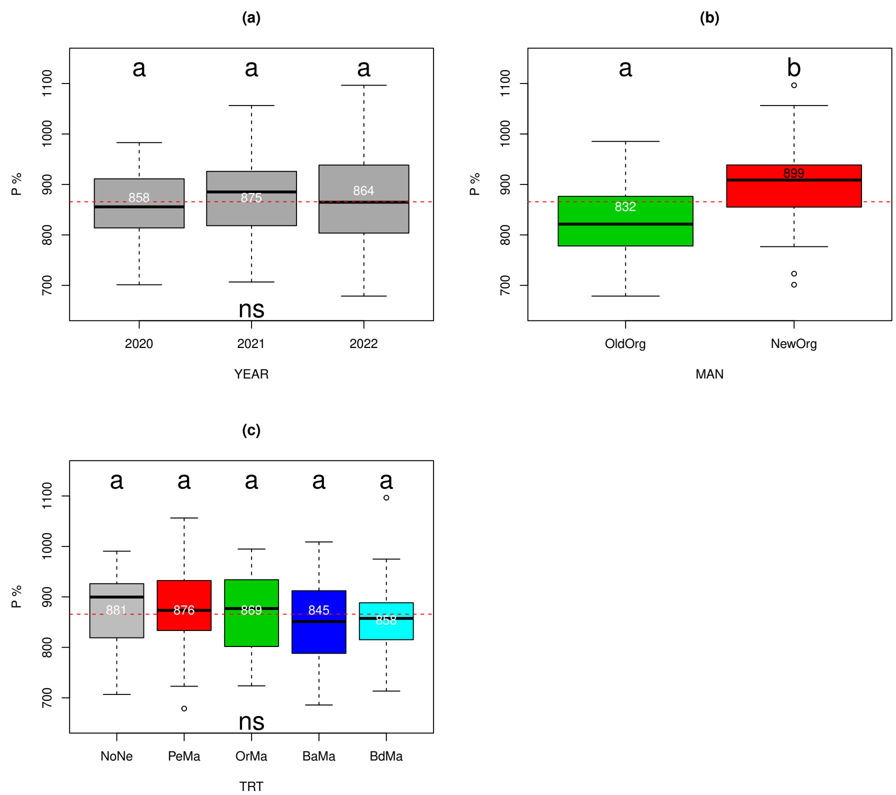


Figure 10: *Total P_2O_5 content during the experiment. No difference were found among TIME (a) and TRTs (c), while NewOrg showed higher P_2O_5 values as compared to OldOrg (b). The abbreviations NoNe, PeMa, OrMa, BaMa and BdMa, i.e TRTs, indicate no amendment, pelleted cow manure, fresh organic cow manure from an organic certified farm, fresh organic cow manure from an organic certified farm added with biodynamic preparations and then composted and cow compost from a biodynamic certified farm, respectively. The abbreviations OldOrg and NewOrg indicate Old Organic and New Organic managed fields (MAN).*

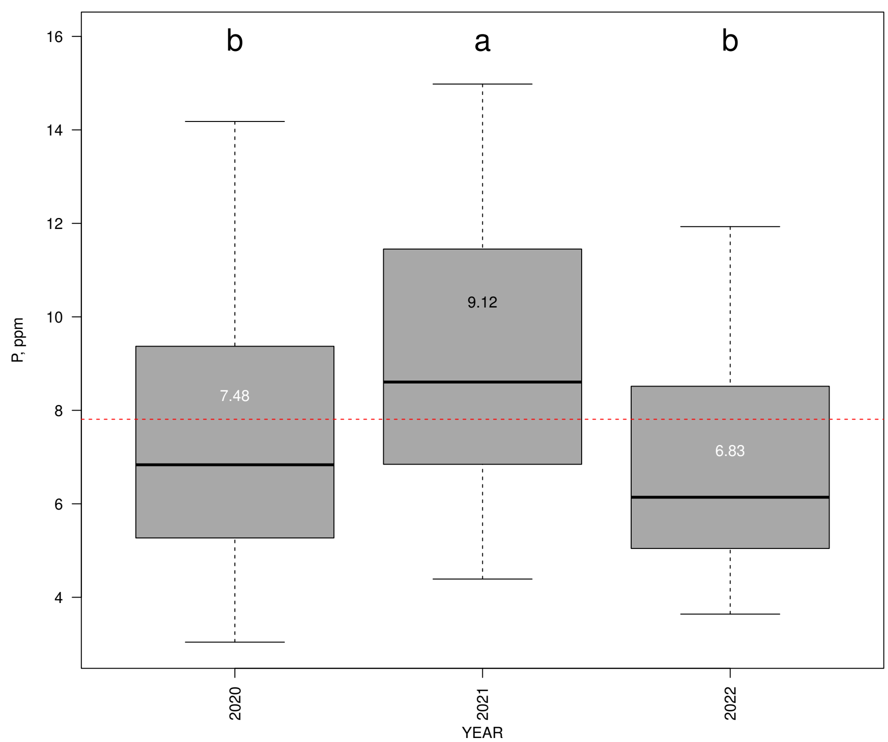


Figure 11: *Available P_2O_5 during the experimental years. A significant increase in 2021 followed by a decrease in 2022 was observed.*

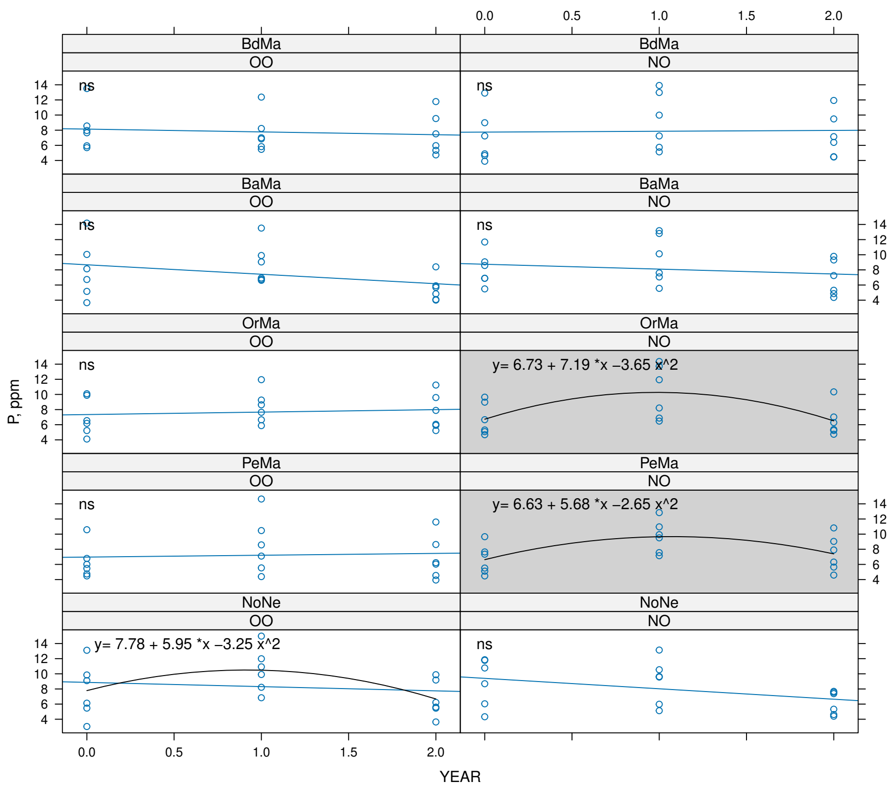


Figure 12: *Available P_2O_5 was assessed using a mixed-effects model. Panels with a grey background indicate statistical significance for the curvatures, i.e. an increase in 2021 followed by a decrease in 2022. The abbreviations NoNe, PeMa, OrMa, BaMa and BdMa, i.e TRTs, indicate no amendment, pelleted cow manure, fresh organic cow manure from an organic certified farm, fresh organic cow manure from an organic certified farm added with biodynamic preparations and then composted and cow compost from a biodynamic certified farm, respectively. The abbreviations OldOrg and NewOrg indicate Old Organic and New Organic managed fields (MAN), respectively*

6.4.1.4 Aggregate Stability The stability of aggregates in soil was compositionally analyzed, sensu Aitchison (1986), since no evidence arose from a customary ANOVA analysis. The aggregate's breakdown among TRTs as a function of time is shown in Figure 13. The colored dots are snapshots of the suspended material for each TRT. The cloud of dots is composed by single sample taken from zero to minute 23. As the time pass by, the composition of suspended particles moves from a coarser composition to a finer one. Solid lines indicate the quadratic relationships between the composition and time. The effect of slaking is evident from the difference in composition between Wet and Dry samples, these last ones being able to produce lower percentages of particles greater than 250 μm at the start of the measure, when the explosive power of trapped air is at its maximum.

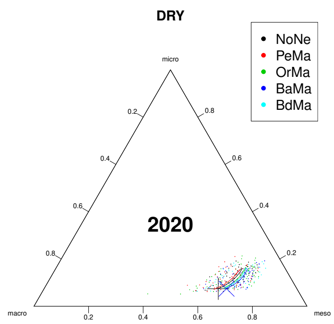
The exploration of model space through comparison of many marginal compositional models (van den Boogaart and Tolosana-Delgado, 2013), allowed us to establish that:

(i) the composition of suspended fractions is quadratically linked to time;

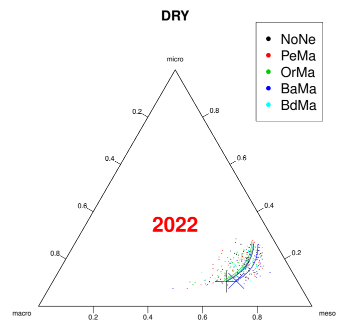
(ii) TRTs exhibited significant heterogeneity before the fertilization (2020) (Figure 13a and c);

(iii) After two fertilization (2022), *BaMa* showed a reduction in aggregate stability under Dry conditions (Figure 13b), while no significant differences under Wet conditions were observed. (Figure 13d).

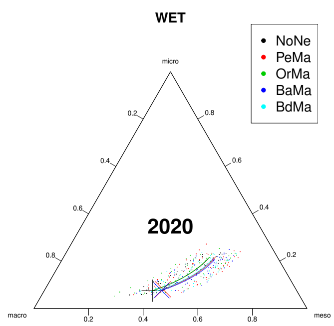
Based on the above considerations, no apparent effect of TRTs on aggregate stability was found. This result is further supported when considering the difference between 2020 and 2022 (before and after the application of TRTs), independent of the TRT variable (Figure 14). After two amendment distributions, soil fragments shift towards smaller diameters indicating a decrease in the toughness of soil cements (Figure 14b).



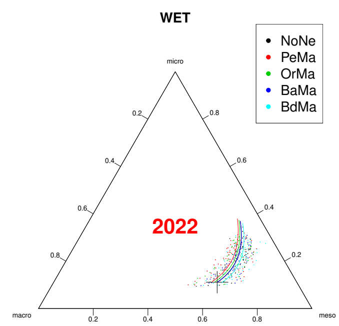
(a) 2020 - before fertilization



(b) 2022 - after two fertilization

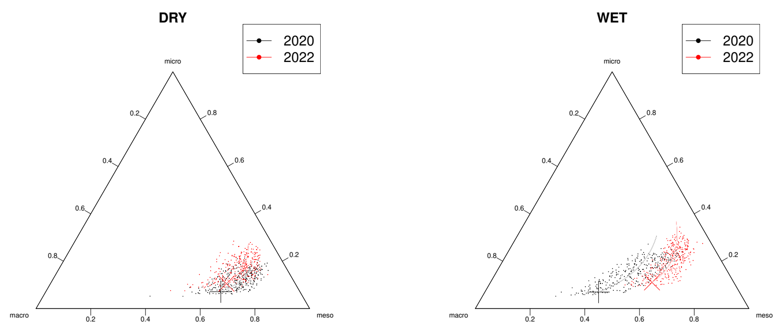


(c) 2020 - before fertilization



(d) 2022 - after two fertilization

Figure 13: *The evolution of aggregate breakdown for the TRT variable is illustrated before (a-Dry and c-Wet, respectively) and after two amendment distributions (b-Dry and d-Wet, respectively). Macro, meso and micro at triangle vertices indicate diameters greater than 250 um, within 250 um and 20 um and smaller than 20 um, respectively. Dry and Wet refer to the humidity of the aggregates. The abbreviations NoNe, PeMa, OrMa, BaMa and BdMa, i.e TRTs, indicate no amendment, pelleted cow manure, fresh organic cow manure from an organic certified farm, fresh organic cow manure from an organic certified farm added with biodynamic preparations and then composted and cow compost from a biodynamic certified farm, respectively. + indicates REF-TRT, i.e. NoNe; × indicates the TRTs with significantly* ($p <= 0.05$) *higher aggregate dimension as compared to REF-TRT.*



(a) *(b)*

Figure 14: *The evolution of aggregate breakdown before and after the two fertilisations in Dry (a) and Wet (b) conditions. Macro, meso and micro at triangle vertices indicate diameters greater than 250 um, within 250 um and 20 um and smaller than 20 um, respectively. Dry and Wet refer to the humidity of the aggregates. The abbreviations NoNe, PeMa, OrMa, BaMa and BdMa, i.e TRTs, indicate no amendment, pelleted cow manure, fresh organic cow manure from an organic certified farm, fresh organic cow manure from an organic certified farm added with biodynamic preparations and then composted and cow compost from a biodynamic certified farm, respectively.* $+$ *and* $\times$ *indicate before (2020) and after two amendments (2022), respectively. In 2022, the soil fragments shift towards significantly smaller diameters (p* $<=$ 0.05*) compared to 2020.*

6.4.1.5 Soil Penetration Resistance Results for soil penetration resistance are presented in Figure 15. Regarding TRTs, a higher compaction in *NewOrg-BdMa* was found. On the contrary, *OldOrg-BdMa* showed a lower compaction compared to the others TRTs. However, these differences also occurred at the beginning of the experiment (month 0). Therefore, only a constant increase in soil compaction was noticed.

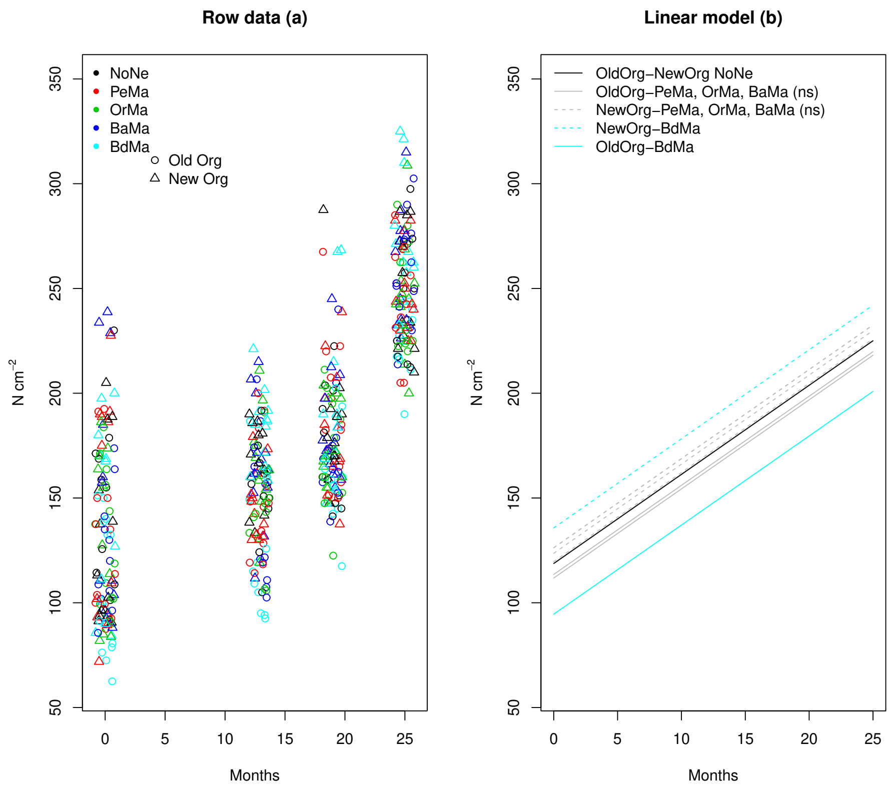


Figure 15: *Soil penetration resistance from the first sampling until the end of the experiment. Row data (a) and the result obtained from a linear model (b) were showed. A general constant soil compaction over time occured (from about 100 N cm^{-2} to 200 N cm^{-2}). The abbreviations NoNe, PeMa, OrMa, BaMa and BdMa, i.e TRTs, indicate no amendment, pelleted cow manure, fresh organic cow manure from an organic certified farm, fresh organic cow manure from an organic certified farm added with biodynamic preparations and then composted and cow compost from a biodynamic certified farm, respectively. The abbreviations OldOrg and NewOrg indicate Old Organic and New Organic managed fields (MAN). Black and coloured lines are TRTs not significantly different ($p <= 0.05$) and significantly different as compared to the REF-TRT, i.e. OldOrg-NoNe), respectively.*

6.4.2 Biological Indicators

6.4.2.1 Soil Microbial Communities The Chao1 index and the Shannon diversity index ranged between 179 and 362, and between 2.35 and 4.35, respectively. However, no differences in *alpha* diversity were observed ($p >= 0.05$). The structure of the microbial communities was not different under the tested conditions, as clearly depicted in the NMDS plot and confirmed by PERMANOVA (Figure 16). The two most abundant genera were *Solicoccozyma* and *Alternaria* (Figure 17). The relative abundance of the genus *Monographella* was higher in *BdMa* compared to *PeMa*, while the relative abundance of the genus *Scutellospora* was higher in *OrMa* compared to *BdMa* (Table 6). These results indicate that TRTs did not produce a significant change in the composition of the fungal communities.

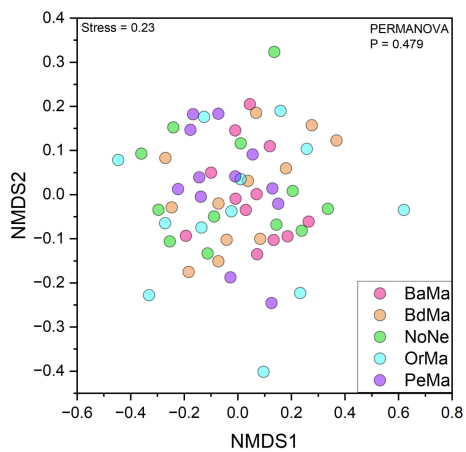


Figure 16: *Non-metric multidimensional scaling for microbial communities. The abbreviations NoNe, PeMa, OrMa, BaMa and BdMa, i.e TRTs, indicate no amendment, pelleted cow manure, fresh organic cow manure from an organic certified farm, fresh organic cow manure from an organic certified farm added with biodynamic preparations and then composted and cow compost from a biodynamic certified farm, respectively.*

Table 6: *Fungal genera with a different relative abundance among the tested conditions. Letters "a" and "b" show significant differences among TRTs ($p <= 0.05$). The abbreviations NoNe, PeMa, OrMa, BaMa and BdMa, i.e TRTs, indicate no amendment, pelleted cow manure, fresh organic cow manure from an organic certified farm, fresh organic cow manure from an organic certified farm added with biodynamic preparations and then composted and cow compost from a biodynamic certified farm, respectively.*

Genus	BaMa	BdMa	NoNe	OrMa	PeMa	p
			%			
Monographella	1.12±0.29 ab	1.90±0.31 a	1.49±0.37 ab	2.05±0.52 ab	0.71±0.11 b	0.038
Scutellospora	0.13±0.13 ab	0.00±0.00 b	0.00±0.00 ab	0.63±0.54 a	0.40±0.39 ab	0.032

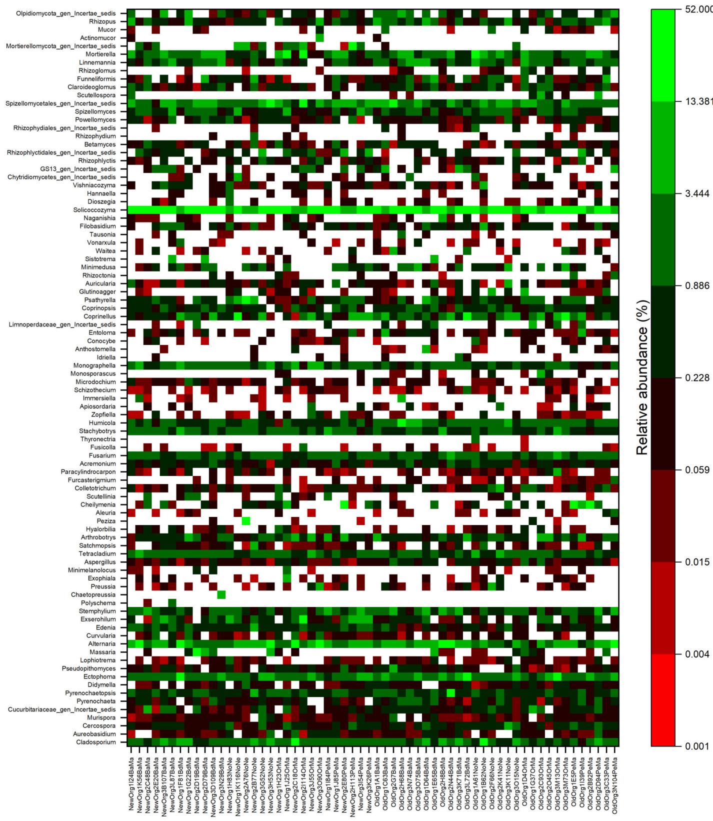


Figure 17: *Relative abundance of the fungal genera. Only the classified genera with a relative abundance of 1%, or higher, are reported.*

6.4.2.2 Earthworms Earthworms data showed the following scenario:

- only 207 out of 600 sampling showed the presence of earthworm individuals, resulting in numerous zero counts in the earthworm sampling records (Figure 18a).
- a subset of samples, containing from 5 to 12 earthworms, deviated significantly from the average earthworm count. These samples were composed by young and baby earthworms and were concentrated in specific areas, probably a spawning/laying site.

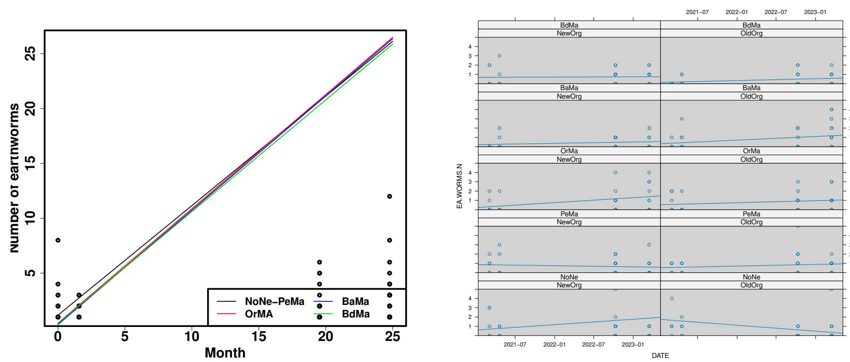
This scenario was addressed through data reparametrization. For each SUB-REP, the success rate of finding at least one earthworm was calculated based on:

(i) complete success, i.e. 1: at least one earthworm for each SUB-REP

(ii) success at 0.25: one earthworm within four SUB-REPs

This method decreased the overall count of zeros and treated samples ranging from 5 to 12 as having a success rate of 1.

In Figure 18 earthworm abundance after data reparametrization is presented. The probability of finding at least one earthworm increased over time, while TRTs and MAN did not show significant differences.



(a) Distribution ***(b)*** Generalized linear model

Figure 18: *Earthworm abundance distribution (a) and earthworm abundance analyzed through a generalized linear model (b). The probability of finding one or more earthworms increase over time. The abbreviations NoNe, PeMa, OrMa, BaMa and BdMa, i.e TRTs, indicate no amendment, pelleted cow manure, fresh organic cow manure from an organic certified farm, fresh organic cow manure from an organic certified farm added with biodynamic preparations and then composted and cow compost from a biodynamic certified farm, respectively. The abbreviations OldOrg and NewOrg indicate Old Organic and New Organic managed fields (MAN).*

6.4.2.3 Abundance and Biomass of Weeds No differences between MAN and TRTs were found. However, an increase in weed biomass over the years was observed (Figure 19a and b, respectively), while weed abundance significant decrease in 2022, when alfalfa was sown (Figure 19c).

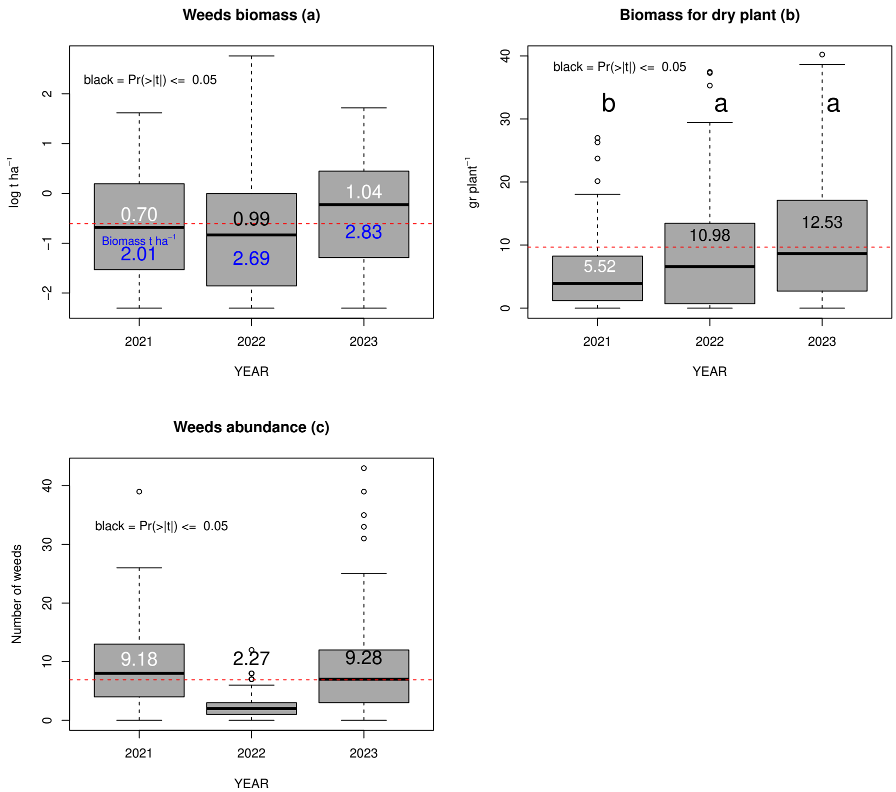


Figure 19: *Weed biomass (a), mean biomass for each weeds individual (b) and weed abundance (c) over TIME.*

6.4.2.4 Yield Yield for ancient common wheat and alphalpha were presented in Figure 20. Ancient common wheat showed significantly higher yields in *NoNe*, *PeMa* and *OrMa* compared to *BaMa* and *BdMa* for both MAN (Figure 20a). On the contrary, alfalfa showed higher yield in *OldOrg-PeMa* compared to the other TRTs (Figure 20b), while no differences were found among TRTs in *NewOrg* (Figure 20c).

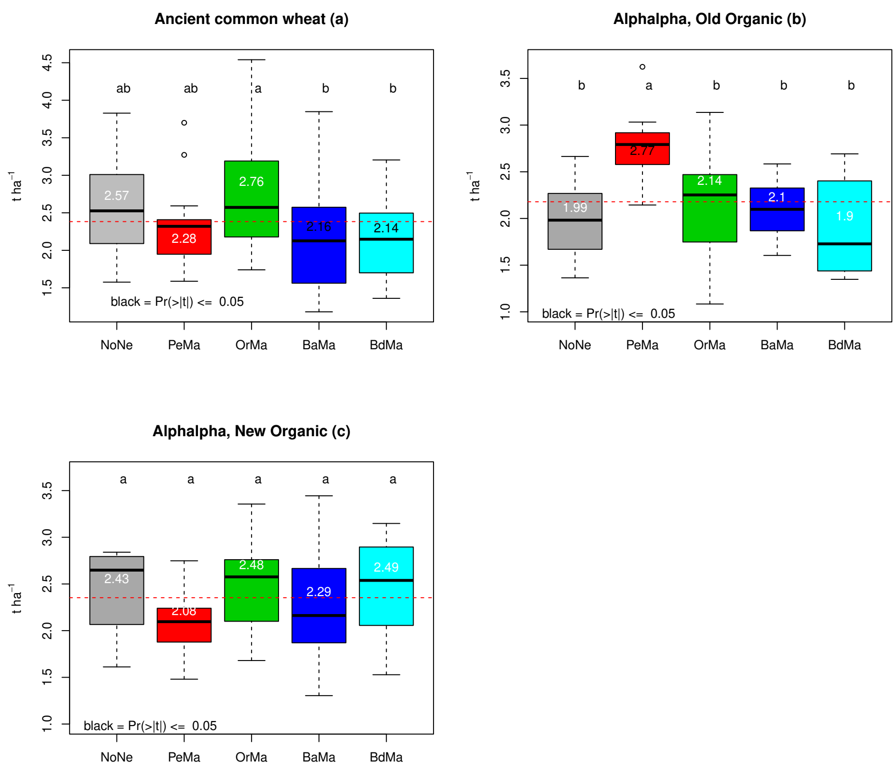


Figure 20: *Yield among treatments (TRTs) for ancient common wheat in both systems (a) and for alfalfa in Old Organic (b) and New Organic (c) systems. The abbreviations NoNe, PeMa, OrMa, BaMa and BdMa, i.e TRTs, indicate no amendment, pelleted cow manure, fresh organic cow manure from an organic certified farm, fresh organic cow manure from an organic certified farm added with biodynamic preparations and then composted and cow compost from a biodynamic certified farm, respectively.*

6.5 Discussion and Concluding Remarks

The aim of this study was to carry out a systemic soil fertility assessment to test different type of organic amendments such as pelleted manure, fresh manure and biodynamic compost.

Chemical indicators did not showed significant differences among TRTs. These indicators showed an increase in values after the TRTs distribution, followed by a subsequent decrease to the initial values. However, the same trend was also noted in the control plot (*NoNe*) where no amendments was applied. The only notable differences were observed in the total P_2O_5 values between MAN. However, these differences might be attributed to a block effect. Therefore, the observed results could be attributed to external factors such as climatic conditions or crop rotation effects.

Regarding physical indicators, no significant differences were observed among TRTs. Generally, the addition of organic matter is anticipated to enhance aggregate stability and reduce soil compaction. Despite the expectation, these effects were not observed in the current study. Again, the observed results may be attributed to external factors such as climatic conditions or crop rotation effects.

Concerning biological indicators, no significant differences were observed among the TRTs. Earthworm abundance increased over time. This increase could be attributed to the crop rotation, which consists in common wheat (2021) followed by alphalpha (2022-2023). As stated by Hoeffner et al. (2021), the introduction of multi-annual species into a crop rotation significantly increased earthworm abundance. This increase could be also linked to the timing of ploughing. As reported in a previous study at MoLTE, ploughing have adversely affected the abundance of earthworms (Pantani et al., 2022). The last ploughing conducted in this study was in August 2022. This result in undisturbed soil during the last earthworm sampling sessions, which may increase their abundance in the soil. It is important to note that ploughing was chosen because it represents the sole tillage operation for incorporating amendments into the soil. Weed abundance decreased in 2022, coinciding with the sowing of alfalfa. On the contrary, weed biomass increased over the years. Therefore, the dynamics of weed species could be linked to the influence of crop rotation.

Regarding crop yield, significant differences were found among TRTs. Nevertheless, a similar effect of TRTs on both ancient common wheat and alfalfa was not evident. Consequently, drawing unambiguous conclusions regarding this indicator poses a challenge.

In conclusion, to date, the tested amendments have not influenced the chemical, physical, and biological fertility of the soil.

Throughout this three-years study (2020-2023), the predominant effects were associated with crop rotation and climatic conditions. Crop rotation is an essential component in organic agroecosystems management, where the established crop rotation was drawn before the starting of this experiment. The climate conditions during the experimental period were characterized by extended period of drought and high temperatures which may have influenced the outcomes of the experiment.

As it is well known, the assessment of soil fertility is a complex matter and requires a long-term perspective for comprehensive evaluation (Pantani et al., 2022). Consequently, another factor which may have affected the results could be the relatively short time-frame of the experiment (3 years).

Future developments entail further analysis of the tested indicators. Moreover, an additional indicator, namely soil microarthropods, will be evaluated in the near future. Data were collected in the 2021-2023 agricultural campaigns and currently being processed. Microarthropods have been demonstrated to respond sensitively to soil management practices (Parisi et al., 2005). Therefore, they could be promising since their potential for a more prompt response to organic amendments.

6.6 Author contribution

Margherita Santoni: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Funding acquisition.

Ottorino-Luca Pantani: Conceptualization, Methodology, Software, Validation, Formal analysis, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision.

Francesco Serafini: Formal analysis, Investigation, Visualization.

Lorenzo Ferretti: Investigation, Funding acquisition.

Carlo Viti: Validation, Writing – review and editing, Supervision.

Matteo Daghio: Formal analysis, Writing – review and editing.

Gaio Cesare Pacini: Conceptualization, Validation, Investigation, Writing – original draft preparation, Writing – review and editing, Supervision, Project administration, Funding acquisition.

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

Margherita Santoni[1], Ottorino-Luca Pantani[1], Francesco Serafini[1], Lorenzo Ferretti[1], Jean-Francois Vian[2], Gaio Cesare Pacini[1]

[1] Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali (DAGRI) - University of Florence (Italy)
[2] ISARA, Lyon (France)

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7.1 Abstract

The current climate, energy and food crises require a reflection on the suitability of agricultural production systems. We analyzed the data collected in the MoLTE field trial, where organic and conventional arable farming systems are running since 1992. Yields significantly decreased with time in both systems (about -79% and -37% since the beginning of the experiment for spring and winter crops, respectively), which is most probably due to the reduced cumulative rainfall from seeding to harvesting. Organic winter crops constantly yielded about 21% less than the conventional ones while spring crops did not show significant differences. The energy use efficiency in organic system was higher than in conventional one. Organic systems could address the current challenges and increase the sustainability of global food systems.

Keyword: organic and conventional agriculture, Mediterranean area, energy balance, climate change

7.2 Introduction

The phrase "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 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 rainfed crops in certain locations, primarily due to more frequent droughts (Ali et al., 2022). 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 sustainable perspective, the current global energy crisis, ultimately defined as a shock of unprecedented breadth and complexity (IEA, 2022), no longer allow the massive use of high-energy inputs such as chemical fertilizers, pesticides, and irrigation. Amidst the growing emergency in energy and climate, understanding which agricultural production system performs better in terms of energy consumption becomes crucial. Several modeling studies have promoted the idea of organic farming being a viable option to face future adverse scenarios, mostly because of its capacity to achieve satisfying levels of food production while consuming less resources (Mäder et al., 2002; Muller et al., 2017; Poux & Aubert, 2018). However, further efforts are needed to understand to what extent organic agriculture can cope with adverse scenarios, given the different pedologic, climatic and agronomic conditions. The inevitable multiple interactions among these factors, over medium and long-term durations and spatial scales, perfectly resume 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 potentially infinite number of components and processes including functional biodiversity, energy flows, biogeochemical cycles, and interactions between organisms and biotopes. Considering these aspects, the ability to evaluate the impact of farming practices becomes overwhelmingly complex. To elucidate these intricate interactions, it is necessary to consider the results from specifically designed Long-Term Experiments (LTE), where the 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 searched within a sustainability choice space (Potschin-Young & 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, scarce levels of water retention, most probably due to low levels of organic matter in soils, often about 1.5% (Altobelli & Piazza, 2022).

Backed by the above listed considerations, we have analyzed the data recorded in the Montepaldi Long Term Experiment - MoLTE[6], the most durable LTE of the Mediterranean area. Organic and conventional production systems were established in 1992 and they are kept running since then. The dataset, covering the period from 1993 to 2022, focuses on grain crops and includes climatic variables (minimum and maximum daily temperature and rainfall), soil parameters, and agronomic details such as fertilizers amounts, tillage operations, sowing and harvesting dates, weeding, yields, etc. 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, here we present the results for the main staple non-irrigated crops such as common and durum wheat, barley, maize, and sunflower, correlating them with rainfall availability and energy use.

[6] https://www.dagri.unifi.it/vp-475-molte.html?newlang=eng and Supplementary Materials

7.3 Results

7.3.1 Agronomic Aspects and Climate Changes Relationships

The relationships between yields and time are presented in Figure 1. Yields significantly decreased over the years in both systems. For winter crops, the decrease 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 of -79% (from 2.4 to 0.5 ton ha^{-1}) in both systems. This marked drop in yields may be attributed to a significant decrease in cumulative rainfall during the winter and spring crops' vegetative cycle (Figure 2). However, we found a significant shift forward in the sowing date for winter crops, which might have contributed to the decrease in rainfall. The winter sowing dates advance by approximately 32 days, from around November 3th in 1994 (DOY 307) to December 5th in 2017 (DOY 339), while no difference was observed for spring crops (Figure 3). In order to exclude the potential impact of the delayed sowing date on the cumulative rainfall outcomes, the same analysis was performed with the winter crops sowing date held constant at the October 25th, representing the earliest date recorded at MoLTE throughout the years. Nevertheless, the results obtained indicate a significant reduction in rainfall (Figure 4). Additionally, an increase of about 1°C in daily maximum temperatures from 1993 to 2022 was estimated (Figure 5).

Since the rate of decrease in winter crops yields is not different for the two systems (common slope at -0.07 ton ha^{-1} per year), the yields can be compared at any time, but it is convenient to use the mean values (intercepts) at 1994. Those values show that organic winter crops yielded 3.8 ton ha^{-1}, while the conventional ones yielded 4.8 ton ha^{-1} (Figure 1), representing a significant -21% lower grain yield. Spring crops, on the contrary, did not show significant differences between the two systems. In general, yields for winter crop at MoLTE were comparable to those in the surrounding areas, while those for spring crops were lower mainly because of the absence of irrigation (data not shown).

Regarding soil parameters, available P_2O_5 decreased over the years both in organic and conventional systems. On the contrary, soil organic matter and total N remained constant (Table 1). 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%).

7.3.2 Energy Balance

The impact of organic and conventional practices on energy balance was assessed through Energy inputs (E, GJ ha^{-1}) and Energy Use Efficiency (*EUE* - Table 2).

The results for E are shown in Figure 6. In the conventional system, an initial marked drop was observed both for winter and spring crops, which is mainly attributed to the reduction of both chemical fertilizers and fuel inputs (data not shown). For winter crops, the conventional E are consistently higher than organic ones, while for spring crops the conventional and organic E were almost the same from 2004 to 2008. This pattern will be elucidated further in Section 4.

The results for *EUE* are presented in Figure 7. A consistent difference is observed between the two systems in both winter and spring crops. Organic winter crops showed a 33% higher *EUE* compared to the conventional counterparts. Even greater efficiency was observed for spring crops, with a 44% higher *EUE* in the organic system. The above described constant difference is associated with a parabolic course along the years, with a common curvature between the two systems. Spring crops, on the other hand, showed a decrease of -0.2 units per year. This apparently low yearly value, after 24 years becomes an efficiency loss of -53% (100-(9+(-0.2x24)/9)x100) and -95% (100-(5+(-0.2x24)/5)x100) for organic and conventional systems, respectively.

7.4 Discussion and Concluding Remarks

The objective of this article was to investigate the agronomic performance in terms of yield and energy use of organic and conventional arable farming systems in the Mediterranean area, presenting the data collected from a 30-year field trial.

Firstly, yields in both systems, for winter and spring crops, significantly decreased over the years (Figure 1). Organic winter crops yielded 21% less than conventional ones, while spring crops did not show significant differences between the two systems.

Climatic, agronomic and, energy data were explored to find some possible explanations for this decrease.

Observing climatic aspects, a substantial decrease in cumulative rainfall during the winter and spring crop cycle was found (Figure 2). Furthermore, a delay in winter crops sowing date was recorded (Figure 3). This probably reflects a decision by agronomists who deemed the climatic and soil conditions unsuitable for sowing in the customary period. It is important to note that the cumulative rainfall would still have decreased over the years even if the seeding had been done without considering climatic and soil conditions, i.e., had been done on a customary date — e.g., October 25th, minimum dataset value — (Figure 4).

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.

Concerning soil parameters, a decrease in available P_2O_5 has been observed over the years in both systems (Table 1). Since that in the organic system the P_2O_5 fertilizers were almost zero all over the years, the decrease in available P_2O_5 is not linked with the organic and conventional management, but probably due to undetermined factors. The P_2O_5 deficiency may therefore be an additional factor that led to the decrease in yield registered at MoLTE (Figure 1). Soil organic matter and total N content remained constant over the years in both systems (Table 1). Therefore, the decrease in yields probably is not determined by these two parameters. The fertilization at MoLTE did not influence the soil parameters in both systems. Since the soil fertility management in stockless systems is challenging, rethink the agronomic techniques adopted at MoLTE are necessary in both systems.

Considering energy aspects, a marked reduction in Energy inputs (E) was observed only in the conventional system (Figure 6). 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 approach later. However, while conventional inputs are consistently higher than organic inputs for winter crops, this pattern is not applicable to spring crops, where conventional and organic inputs were almost the same from 2004 to 2008. The rise in E in organic system during those years, is most probably due to the green manure introduced to fertilize the following maize crop. This is confirmed by the subsequent decrease of organic inputs when green manure was removed.

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 Energy Use Efficiency (EUE) may be of significant relevance (Figure 7). The organic system undoubtedly exhibited better performance in terms of EUE compared to conventional system. This result is consistent with other publications on organic system (Alonso & Guzmán, 2010; Ferro et al., 2017; Mäder et al., 2002). However, some authors share the concern that an increase in cultivated area is needed considering the lower productivity per hectare of organic farming (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).

Based on the above considerations, three main conclusions can be drawn:

- Conventional yields decrease could be attributed to the reduced rainfall, the decrease in P_2O_5, and the reduction in E.
- Organic yields decrease could be solely attributed to the reduced rainfall and the decrease in P_2O_5.
- The organic system, despite the lower yield, showed a higher EUE compared to the conventional one.

Certainly, other factors may have contributed to the decrease in yield. Field observations, devoid of supporting data, prompt us to posit that the decline in yields may be attributed also to the effects of weed competition coupled with the influence of wild animals. Other factors such as pests and diseases likely had a negligible impact on yield, as their prevalence was not significantly observed in the surveyed area.

Spring crops experienced a significant drop in both systems, reaching nearly zero production. This decrease can be attributed to the above mentioned recorded and unrecorded factors. A recorded factor that probably contributed to the drop in spring crops yield may be the introduction of maize in rotation from 2003 to 2009, when rainfall tended to decrease. As maize have a high water requirement, it may have suffered from the lack of water in both organic and conventional systems. Consequently, the cultivation of these crops was discontinued, a trend that was observed also in the surrounding area.

Climatic changes in the Mediterranean area may continue to impact on crop productivity in the next few years. 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). Additionally, 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 5). These strategies are designed considering the upward trend in production costs in near the future.

In conclusion, the farming sector in the Mediterranean area is facing climatic, energy, and food crises. In the face of increasing climate change impacts and amid the ongoing long-forecasted energy crisis, organic system showed a higher Energy Use Efficiency. Therefore, organic management could serve as a viable alternative to mitigate the impact of the global food system on present challenges while enhancing the overall sustainability of human activities on Earth.

7.5 Supplementary Materials

7.5.1 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 over all the Mediterranean area for its duration and amount of data collected. The field experiment encompasses a slightly sloping surface of about 15 hectares. Each individual plot measures 1.3 hectares, with a total of 10 plots. The main soil physico-chemical characteristics at MoLTE are shown in Table 3. 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 were 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 the organic and conventional systems abstain from disease and pest control measures, consistent with ordinary farms in the region. The sole method of protection carried out was seed treatment, using copper in the organic system and fungicides in the conventional one. Additional agronomic details can be found in Table 4. To minimize the risk of interactions and cross-contaminations, natural and artificial hedges have been interposed between the two systems since 1992. Crop rotations, outlined in Table 5, 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. Therefore, 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 6. This table also indicates the number of yield observations for each crop over the years. Furthermore, average yields for each crop estimated by a linear model is presented in Table 7.

7.5.2 Statistical Analyses

The statistical analyses were performed using R statistical software version 4.3.2 (R Core Team, 2023) and several of its libraries (Baker & Mortlock, 2023; Dowle

& Srinivasan, 2023; Fox et al., 2023; Mayer, 2023; Ryan & Ulrich, 2023; Sarkar, 2008, 2023; Sax, 2023; Spinu et al., 2023; Wickham, 2023). Linear models were built using the lm() function. The model space was explored by comparing marginal models. The analysis began with a saturated model, which was refined by removing descriptors until further simplification was not permitted. The analysis of residuals from the final model did not reveal significant deviations from normality.

7.5.3 Climate

The experimental site is characterized by a typical Mediterranean and Sub-Appennine 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 1), while those from 2015/05/21 to 2022/11/20 were selected from 2).

Figure 5 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 were recorded.

7.5.4 Soil Parameters

From 1994 to 2017, the following chemical indicators were measured at MoLTE: soil organic matter (Walkley & Black, 1934), total N content (Kjeldahl, 1883), available P_2O_5 (Olsen et al., 1954), and exchangeable K_2O. In Table 8, the number of observations for the main soil chemical analysis in organic and conventional systems were showed.

7.5.5 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 2. 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 9).

7.6 Figures

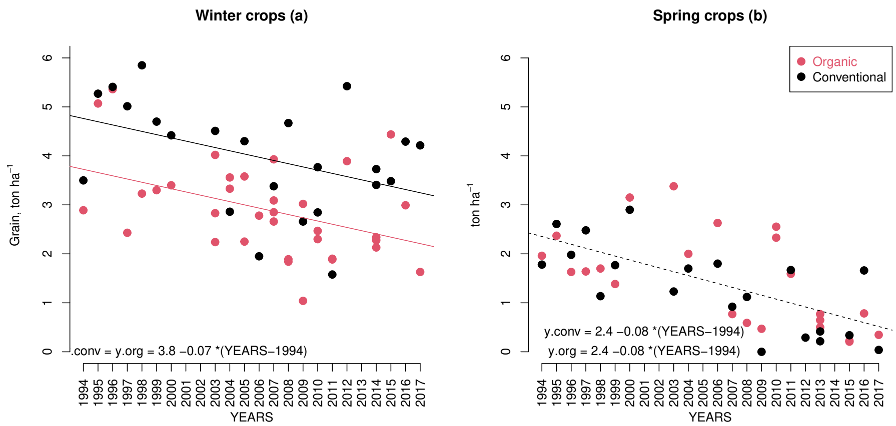

Figure 1: *Yields in the organic and conventional systems for winter (a) and spring (b) crops at MoLTE. Only the crops cultivated simultaneously in both systems were considered. Dotted line represents no significant difference between systems (p >= 0.05).*

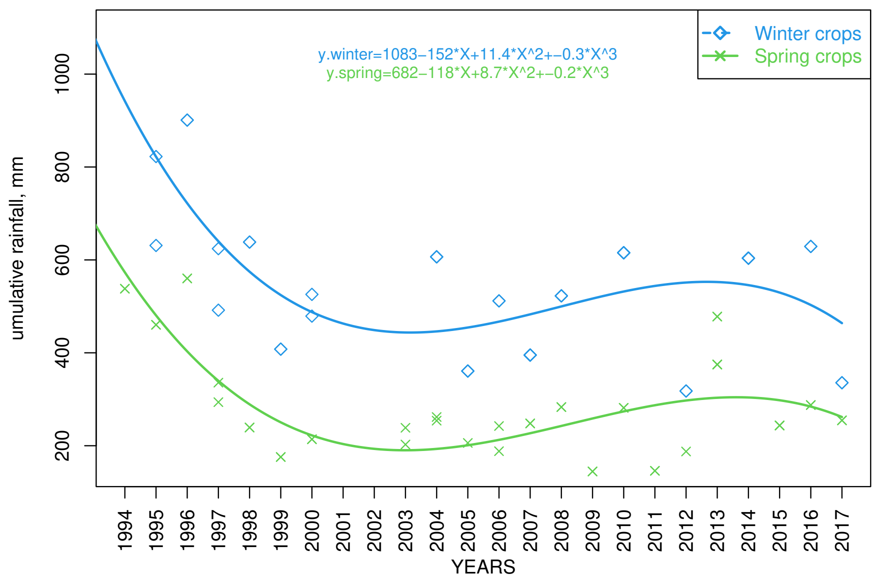

Figure 2: *Cumulative rainfall (mm) during the vegetative cycle of winter and spring crops at MoLTE. X stands for* $YEARS - 1994$. *Only the crops cultivated simultaneously in organic and conventional systems were considered.*

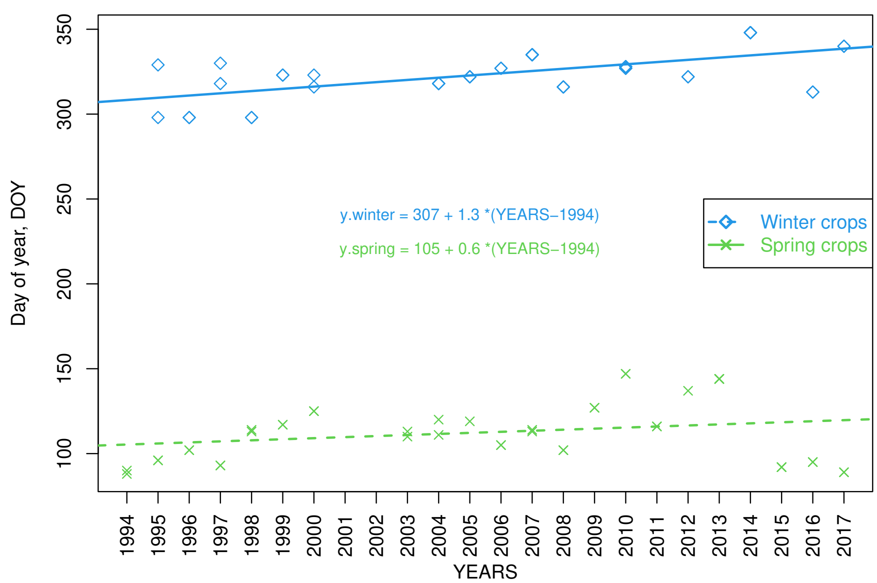

Figure 3: *Sowing dates for winter and spring crops at MoLTE. Only the crops cultivated simultaneously in organic and conventional systems were considered. Dotted line represents no significant difference in sowing dates (p >= 0.05).*

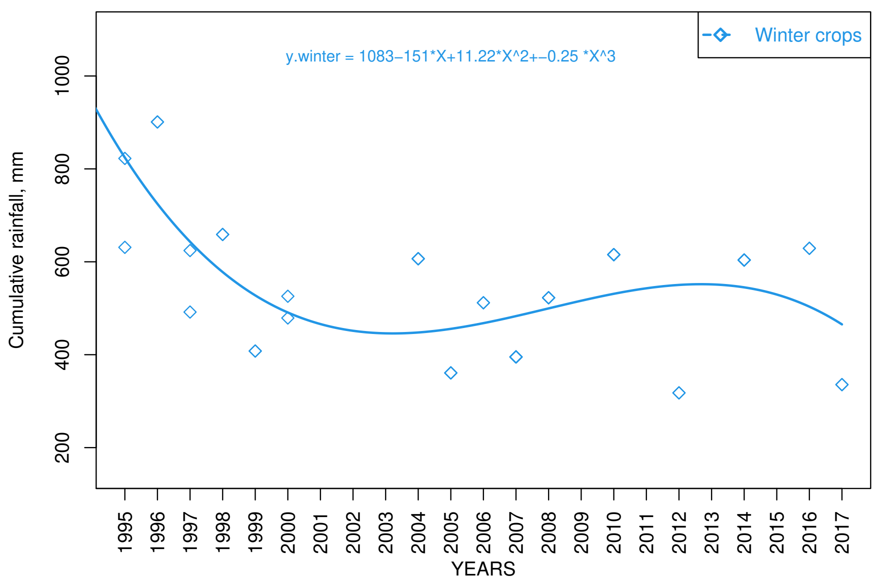

Figure 4: *Cumulative rainfall (mm) during the vegetative cycle of winter crops, keeping constant the sowing date, i.e. the earlier sowing dates recorded at MoLTE all over the years, which were October 25th.* X *stands for* $YEARS - 1994$*. Only the crops cultivated simultaneously in organic and conventional systems were considered.*

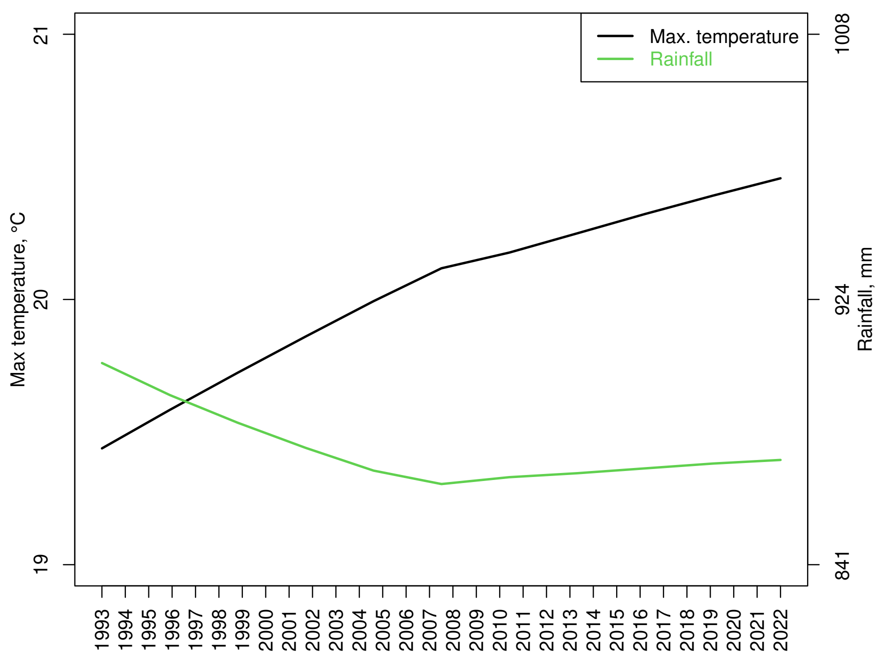

Figure 5: *Trends in maximum temperature (°C) and rainfall (mm) from 1993 to 2022, obtained from MoLTE data and processed using 'stl' function. An increase in maximum temperatures by 1°C and a decrease in rainfall by -166 mm were recorded.*

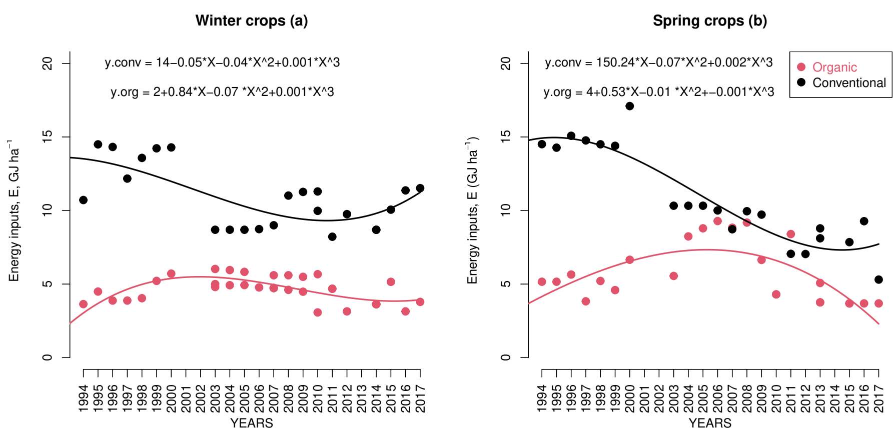

Figure 6: *Energy inputs (E) in the organic and conventional systems for winter (a) and spring (b) crops at MoLTE. X stands for* $YEARS - 1994$*. Only the crops cultivated simultaneously in both systems were considered.*

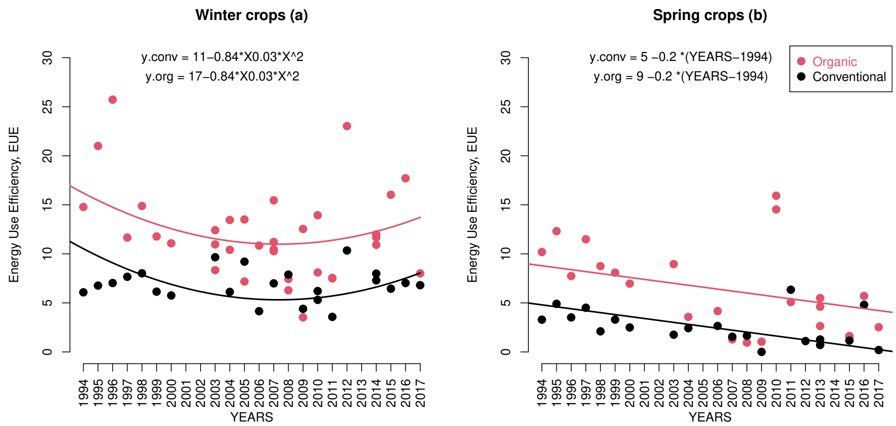

Figure 7: *Energy Use Efficiency (EUE) in the organic and conventional systems for winter (a) and spring (b) crops at MoLTE. X stands for* $YEARS - 1994$*. Only the crops cultivated simultaneously in both systems were considered.*

7.7 Tables

Table 1: *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 (%)	$= 1.6 \times CONV + 0.15 \times ORG$
Total N (g kg^{-1})	ns
Available P_2O_5 (mg kg^{-1})	$= -25.8 \times YEAR + 0.65 \times YEAR^2$

Table 2: *Definitions of energy parameters.*

Energy parameter	Definition	Unit
Direct energy input (Ed)[a]	Input of diesel[b]	GJ ha^{-1} y^{-1}
Indirect energy input (Ei)[c]	Seed + mineral and organic fertilizers + herbicides + machines[d]	GJ ha^{-1} y^{-1}
Energy output (Eo)[e]	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

[a] Energy used within the farm. [b] Total diesel consumption (l ha^{-1}) for the various farm operations. [c] Energy used outside of the farm for the manufacture, packaging and transportation of seeds, fertilizers, pesticides and machines.
[d] Manufacture and maintenance of machinery were determined for each agronomic operation.
[e] The energy content in crop production (harvested products). The non-harvested biomass (e.g. straw, residues and green manure) is not accounted for.

Table 3: *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)	8.30	8.3
C.E.C. (meq. 100 g^{-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 4: *Ordinary tillage operations, fertilization and weeding at MoLTE. During the 30 years of MoLTE experiment, ordinary agronomic operations could change due to year-specific production and climatic condition.*

Crop type	Winter crops		Spring crops	
	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	Di-ammonium phosphate	Green manure or organic fertilizer	NPK: 20.10.10
First fertilization	-	Ammonium nitrate	-	Ammonium nitrate
Second fertilization	-	Urea	-	Urea
Chemical weeding	-	Herbicides[a]	-	Herbicides[b]
Mechanical weeding	Weed harrowing	Weed harrowing	Weed hoeing	Weed hoeing
Disease control	-	-	-	-
Insect control	-	-	-	-

[a] 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%)
[b] GOAL (a.i. oxyfluorfen)

Table 5: Crop rotations from 1992 to 2022 at MoLTE.

	Organic	Conventional
1992-2000	1-Sunflower 2-Faba bean 3-Winter common wheat/winter barley 4-Clover	1-Sunflower 2-Winter common wheat or winter barley
2000-2004	1-Green manure+maize 2-Faba bean 3-Winter barley 4-Clover	1-Maize 2-Winter barley
2004-2008	1-Green manure+maize 2-Faba bean 3-Winter durum wheat/winter common wheat 4-Clover	1-Maize 2-Winter durum wheat or winter common wheat
2008-2013	1-Sunflower 2-Faba bean 3-Winter durum wheat/winter common wheat 4-Alphalpha	1-Sunflower 2-Winter durum wheat or winter common wheat
2013-2019	1-Sunflower/green manure+millet 2-Chickpea/lentil 3-Winter barley or ancient winter common wheat 4-Clover	1-Sunflower 2-Winter barley
2019-2024	1-Ancient winter common wheat 2-Spelt 3-Alphalpha 4-Alphalpha	1-Alphalpha 2-Alphalpha

Table 6: *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 7: *Linear models for the average yields (ton 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)	$= 3.81 \times CONV - 0.92 \times ORG$
Winter common wheat (WC)	$= 2.7 \times CONV - 0.42 \times ORG$
Winter durum wheat (WD)	ns
Maize (MA)	$= -0.29 \times YEAR$
Sunflower (SU)	$= -0.07 \times YEAR$

Table 8: *Number of observations for the main soil chemical analysis from 1994 to 2017 in organic and conventional systemat 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 9: *Energy equivalents used for inputs and outputs at MoLTE.*

	Energy equivalents (MJ unit^{-1})	References
Inputs		
Machinery operations		
Diesel fuel (l)	39.6	Hülsbergen et al. *(53)*
Machines (kg)	Depending on soil tillage operations	Data collected at MoLTE
Mineral and organic fertilizers (kg)		
N	35.3	Hülsbergen et al. *(53)*
P_2O_5	15.8	Hülsbergen et al. *(53)*
Manure	0.3	Dal Ferro et al. *(57)*
Herbicides (kg)	288	Hülsbergen et al. *(53)*
Seed (kg)		
Winter wheat	5.5	Hülsbergen et al. *(53)*
Winter barley	5.5	Hülsbergen et al. *(53)*
Sunflower	12	Lin et al. *(54)*
Maize	14.6	Simon et al. *(56)*
Grain yield output (kg)		
Winter wheat	18.6	Hülsbergen et al. *(53)*; Migliorini et al. *(55)*
Winter barley	18.6	Hülsbergen et al. *(53)*; Migliorini et al. *(55)*
Sunflower	26.8	Lin et al. *(54)*
Maize	14.7	Dal Ferro et al. *(57)*; Migliorini et al. *(55)*

7.8 Author contribution

Margherita Santoni: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization.

Ottorino-Luca Pantani: Methodology, Software, Validation, Formal analysis, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision.

Francesco Serafini: Investigation, Writing – review and editing, Visualization.

Lorenzo Ferretti: Investigation.

Jean-Francois Vian: Validation, Writing – review and editing, Supervision.

Gaio Cesare Pacini: Conceptualization, Validation, Investigation, Writing – original draft preparation, Writing – review and editing, Supervision, Project administration, Funding acquisition.

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8 Supplementary Materials Chapter: FunBies, a Model for Integrated Assessment of Functional Biodiversity of Weed Communities in Agro-ecosystem

Gaio Cesare Pacini[1], Piero Bruschi[1], Lorenzo Ferretti[1], Margherita Santoni[1], Francesco Serafini[1], Tommaso Gaifami[1]

[1] Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali (DAGRI) - University of Florence (Italy)

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8.1 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.

Keyword: Functional Biodiversity, Integrated assessment, Functional traits, Weed community, Organic farming

8.1.1 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

8.2 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 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 & 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 & 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" by Grime (1998), there is new evidence for more complex effects of heterogeneous trait values between species (i.e. "functional divergence hypothesis" or "niche complementarity hypothesis")

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.

8.3 Material and Methods

8.3.1 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 4.

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).

8.3.2 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 5).

8.3.3 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 minor* 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. alexandrinum* 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 & 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 & 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 & Warwick, 2001). Such choice was handled with a heuristic procedure through a subjective inspection of the CA dendrogram (Köbrich et al., 2003).

8.3.4 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.

8.3.5 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 7 combined with figures resuming the LAM model.

8.3.5.1 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 (W. 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.

8.3.5.2 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.

8.3.5.3 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 & 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.

8.3.5.4 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.

8.3.5.5 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.

8.3.5.6 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).

8.3.5.7 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).

8.3.5.8 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).

8.3.5.9 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 & Moonen, 2014; Gabriel & Tscharntke, 2007; Gibson et al., 2006; W. 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 & Carr, 2005; Mitchell et al., 2004; Molina-Montenegro & 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:

$$\textit{Pollinator attractiveness} = \textit{Müller class score} * 0.7 * \textit{Flowering period score} * 0.3 \quad (4)$$

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.).

8.3.5.10 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 bio-control 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 bio-control 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).

8.3.5.11 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 & Poesen, 2003; G. 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.

8.3.5.12 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.

8.3.5.13 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.

8.3.5.14 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.

8.3.5.15 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.

8.3.5.16 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.

8.3.5.17 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.

8.3.5.18 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

$$\textit{Cultural heritage value} = \textit{Knowledge score} * 0.5 + \textit{Use score} * 0.5 \tag{5}$$

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 & 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. $0.0 < x < 0.2 = 0.1, 0.2 < x < 0.4 = 0.3, 0.4 < x < 0.6 = 0.5, 0.6 < x < 0.8 = 0.7, 0.8 < x < 1 = 0.9$.

8.3.6 Integrated Assessment of Functional Biodiversity

8.3.6.1 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:

$$FBI = \sum_{Sp=1}^{N} \sum_{ES=1}^{4} w_{ES} * \left[\sum_{EF=1}^{x} w_{EF} * \left(\sum_{FT=1}^{n} w_{FT} * A_{Sp} * S_{FT} \right) \right] \quad (6)$$

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 & 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 7, 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 12.

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.

8.3.6.2 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 1.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 2).

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.

8.4 Results

8.4.1 Selection of Most Representative Crop-weed Communities

In Figures 1, 2, 3, 4, 5, 6 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 2, 4 and 6), 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 2, 4 and 6 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 2), 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 4), 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 6), 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.

8.4.2 Characterization of the Most Representative Crop-weed Communities

In Table 3 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.

8.4.3 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.

8.4.4 Results of the Overall Functional Biodiversity Index at System Level

In Figure 8 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.

8.4.5 Provision of Ecosystem Services per Macro-group of Crop-weed Communities

In Figures 9 and 10 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, bio-control, 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 3, 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 11 and Appendix D, Table 11, 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.

8.4.6 Results of the Functional Biodiversity Index at Species Level

In Figure 11 results of the application of FunBies at species 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.

8.5 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.

8.5.1 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.

8.5.2 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).

8.5.3 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. 7).

8.5.4 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. 8), FBI calculated at crop category level (WC+LF and RC+LG, Figs. 9 and 10, respectively) and FBI calculated at species level (Appendix D, Table 11).

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 4). 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 8, 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. 9), 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. 11 and Appendix C, Table 10).

Agroecosystems dynamics are overwhelmingly complex and, indeed, results of ESs for RC+LG crops are reverted as compared to WC+LG(Figs. 10 and 9, 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 3, 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.

8.6 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.

8.6.0.1 Author contribution

Gaio Cesare Pacini: Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Supervision, Data Curation.
Piero Bruschi: Investigation, Supervision.
Lorenzo Ferretti: Investigation.
Margherita Santoni: Investigation, Writing - Review & Editing, Visualization.
Francesco Serafini: Writing - Review & Editing, Visualization.
Tommaso Gaifami: Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original Draft, Data Curation.

8.7 Tables

Table 1: *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 2:** 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 3:** 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

Species	Average abundance	Average similarity	Contribution to group similarity (%)	Cumulative contribution (%)
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
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
Lolium perenne L.	1.2	0.5	2.9	88.0

Species	Average abundance	Average similarity	Contribution to group similarity (%)	Cumulative contribution (%)
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)				
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

Species	Average abundance	Average similarity	Contribution to group similarity (%)	Cumulative contribution (%)
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
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

Species	Average abundance	Average similarity	Contribution to group similarity (%)	Cumulative contribution (%)
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
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

8.8 Figures

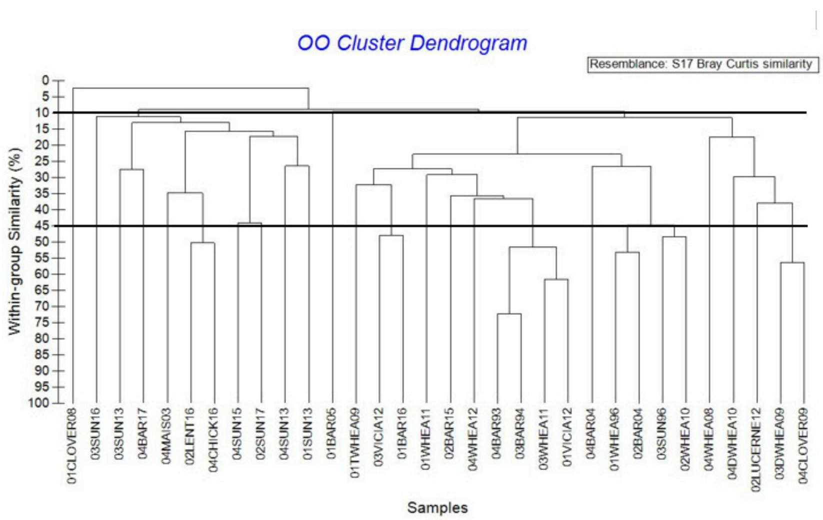

Figure 1: *Cluster dendrogram grouping sample crop-weed communities of the old organic (OO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Valdipesa, Florence, Tuscany, in the period 1993-2017. Results were obtained after standardization by percentage of the species variables and 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.*

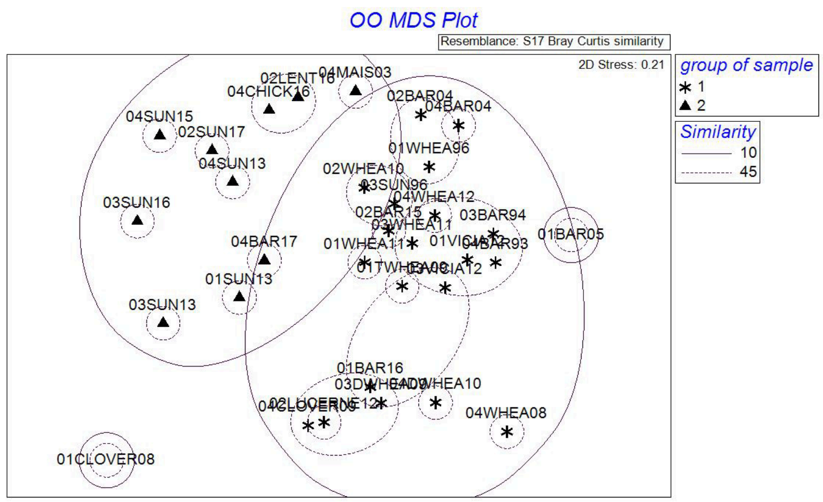

__Figure 2:__ Superimposition of cluster groupings on the multi-dimensional scaling plot representing crop-weed communities of the old organic (OO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Valdipesa, Florence, Tuscany, in the period 1993-2017. Results were obtained after standardization 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.

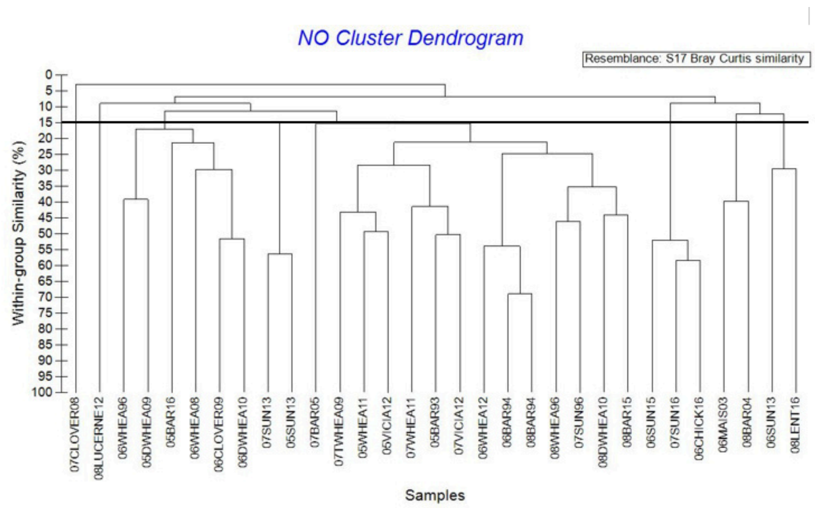

Figure 3: *Cluster dendrogram grouping sample crop-weed communities of the new organic (NO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. Results were obtained after standardization by percentage of the species variables 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.*

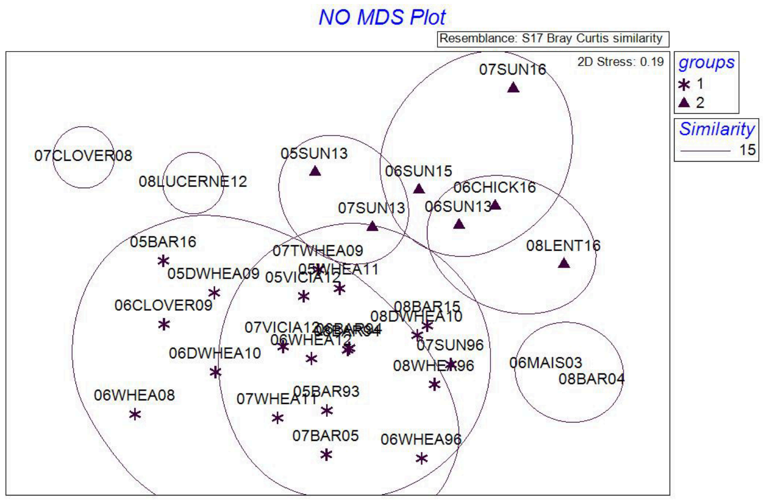

Figure 4: *Superimposition of cluster groupings on the multi-dimensional scaling plot representing crop-weed communities of the new organic (NO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. Results were obtained after standardization 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.*

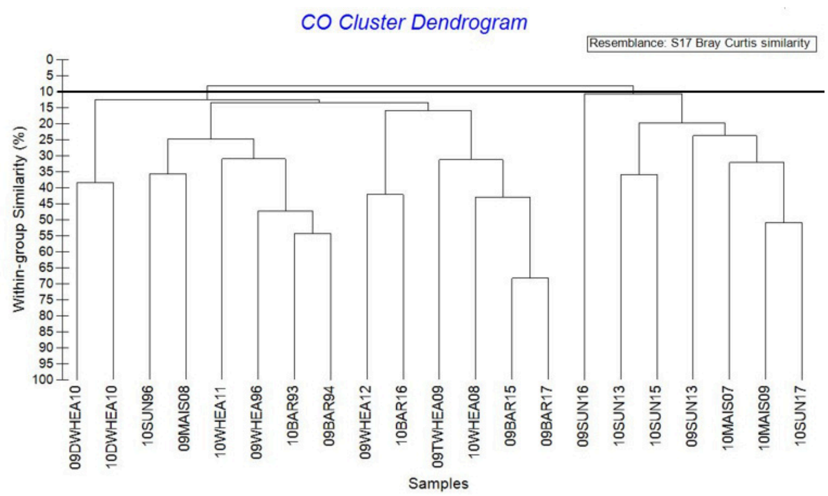

Figure 5: *Cluster dendrogram grouping sample crop-weed communities of the conventional (CO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. Results were obtained after standardization by percentage of the species variables 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.*

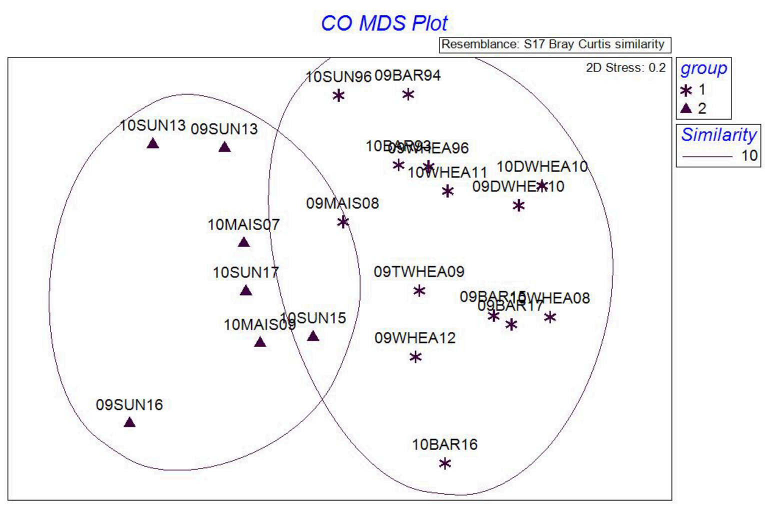

Figure 6: *Superimposition of cluster groupings on the multi-dimensional scaling plot representing crop-weed communities of the conventional (CO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. Results were obtained after standardization 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.*

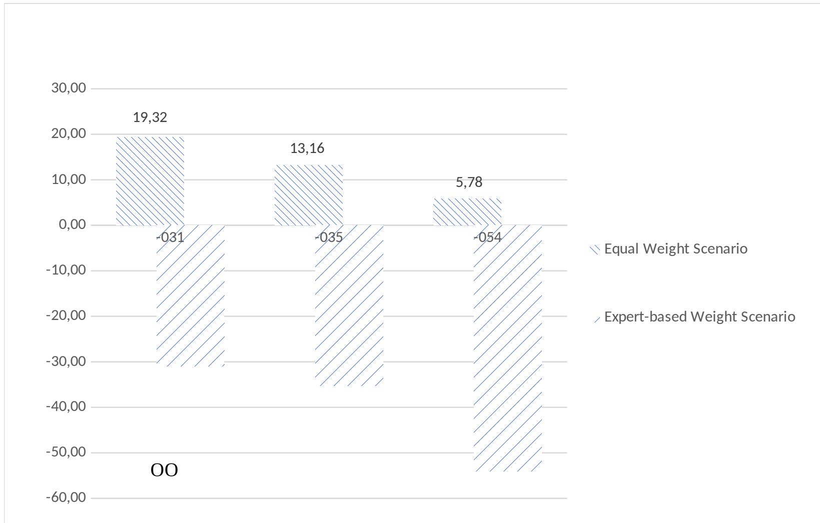

Figure 8: *Yearly averages of overall ecosystem service (ES) provision provided by representative crop-weed communities of the old organic (OO), new organic (NO) and conventional (CO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. 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.*

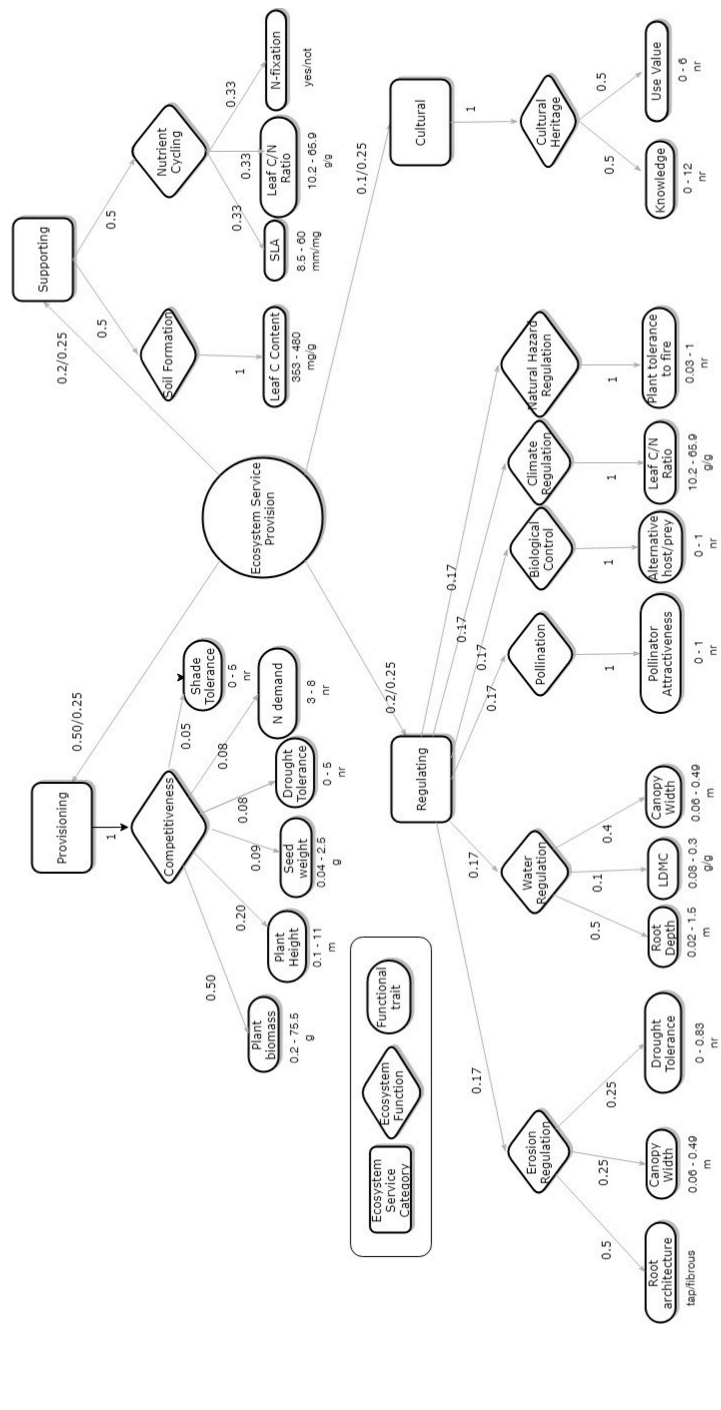

Figure 7: *FunBies model structure. FunBies was applied to each species of the most characterizing weeds of organic and conventional micro-agroecosystems at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany. For each ecosystem service (ES) category (rectangles), corresponding 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.*

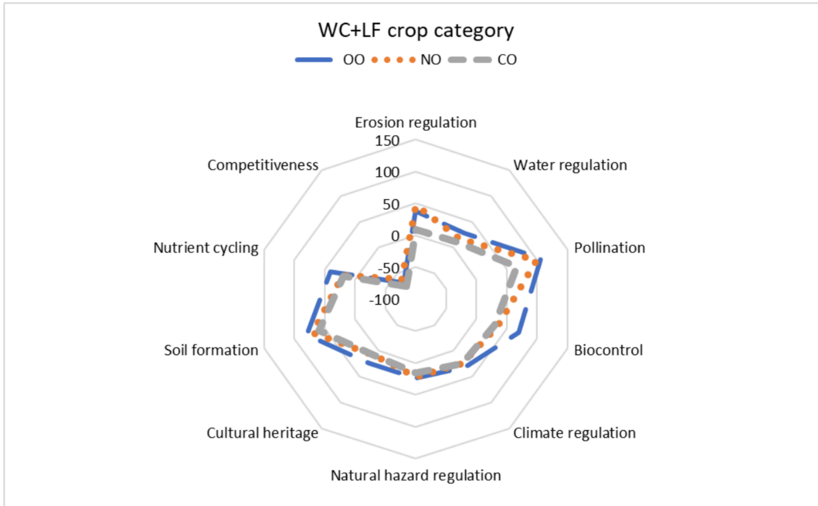

Figure 9: *Provision of ecosystem services by representative crop-weed communities of winter crops + legume crops for forage (WC+LF) category groups in the old organic (OO), new organic (NO) and conventional (CO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, 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.*

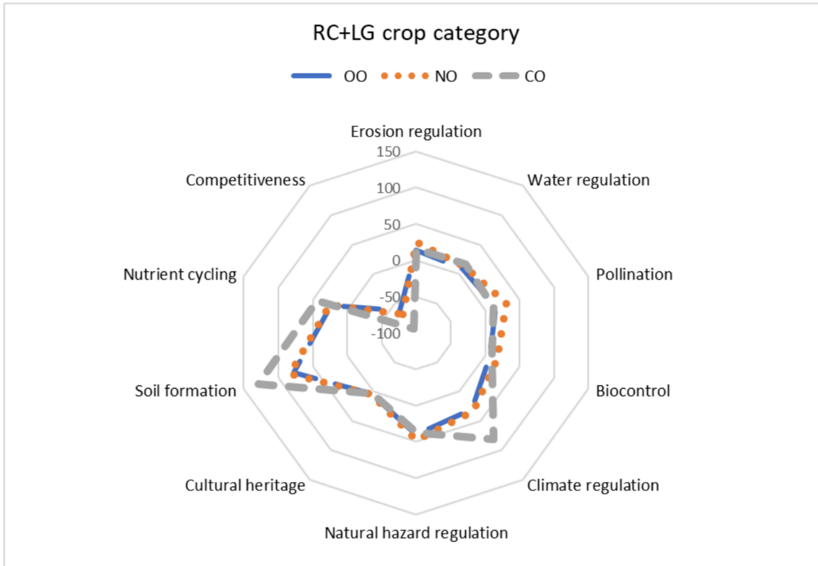

Figure 10: *Provision of ecosystem services by representative crop-weed communities of row crops + legume crops for grain (RC+LG) category groups in the old organic (OO), new organic (NO) and conventional (CO) micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, 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.*

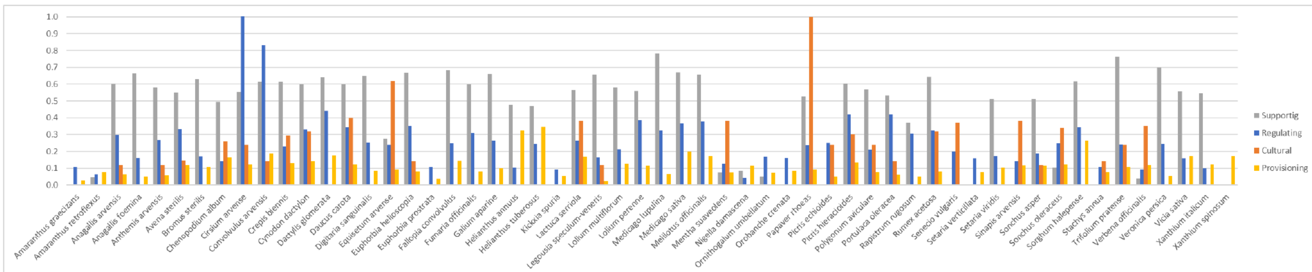

Figure 11: *Supporting, regulating, cultural and provisioning services supplied by each single species collected in the old organic, new organic and conventional micro-agroecosystem at Montepaldi long term experiment, San Casciano Val di pesa, Florence, Tuscany, in the period 1993-2017. The species scores 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.*

Appendices

Appendix A

Table 4: *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.

Table 5: *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.

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 6: *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 7: *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 (CO2 fixed into the soil) instead of emitting CO2 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 8: *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 9: *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 10: *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
Po	pollen flowers	short tongued bees, syrphids, flies, beetles	0.3
W	wind flowers	-	0.0
Wb	wind flowers occasionally 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

Müller Class[1]	Characteristic[1]	Typical Pollinators[1]	Score[2]
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
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, butterflies	0.2

Müller Class[1]	Characteristic[1]	Typical Pollinators[1]	Score[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
PoDe	transition type pollen flowers - nasty flowers	short tongued bees, syrphids, muscids, beetles	0.3
PoWb	transition type pollen flowers - wind blossoms occasionally visited by insect	short tounged bees, syrphids, muscids, beetles	0.3

[1] Durka(2002)
[2] Expert-based.

Appendix D

Table 11: *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
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

Species	Provisioning	Regulating	Supporting	Cultural	FBI
Euphorbia helioscopia L. subsp. *helis-copia*	-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
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

Species	Provisioning	Regulating	Supporting	Cultural	FBI
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
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
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

Species	Provisioning	Regulating	Supporting	Cultural	FBI
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 (6) in the manuscript text, where the species summation component was deleted. The modified equation is reported below.

$$FBI_{Sp} = \sum_{ES=1}^{4} * \left[\sum_{EF=1}^{x} * \left(\sum_{FT=1}^{n} w_{FT} * A_{Sp} * S_{FT} \right) \right] \tag{7}$$

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 12: *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. 7. 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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9 Main Conclusions

The main objective of this research was to carry out a systemic soil fertility assessment to asses organic and biodynamic agriculture as alternative methods to high-input agriculture in a long-term experiment in the Mediterranean region. To achieve this objective, tree phases were identified in the research project:

- To carry out a systemic soil fertility assessment through a wide range of indicators regarding chemical, physical and biological soil properties.
- To assess alternative agronomic techniques aimed at improving soil fertility through practices that reconnect crop and animal production, thereby allowing the local unfolding of nutrient element cycles.
- To provide a 30-year comprehensive analysis in a long-term experiment comparing organic and conventional agriculture, including climatic, agronomic, and soil parameters.

The following conclusions can be drawn from the results of this thesis:

- Soil fertility assessment suggests that organic management positively affects soil biological activity and soil penetration resistance along soil profile; therefore, organic agriculture seems capable of causing long-lasting soil fertility. In conventionally managed fields, high crop yield, possibly linked to higher P_2O_5 availability supplied by synthetic-chemical fertilizers, might lead to a greater aggregate stability.
- Reduced tillage yields harder soils, though it has a positive effect on soil biological properties. In heavy soils subject to dry summer seasons, chisel plowing appeared to be the most balanced tillage option in terms of biological activity and quality of physical structure.
- Soil fertility assessment suggests that, among the measured chemical, physical and biological indicators for describing the state of soil fertility, available P_2O_5, aggregate stability, soil penetration resistance, time-related earthworm abundance, root distribution and yields are the most informative indicators on the impact of management in the MoLTE experiment.
- The organic system showed higher microbial abundance and activity compared to the conventional system. Moreover, the organic system had significantly higher bacterial richness than the conventional system. No significant differences were found in terms of NH_4^+ and NO_2^- contents between the two systems, while a higher soil CO_2 emission and lower NO_3^- were observed in the organic system.
- Alternative fertilization techniques using pelleted manure, fresh manure and biodynamic compost have been assessed to improve soil fertility in organic systems. However, to date, the tested fertilizers have not influenced the chemical, physical, and biological fertility of the soil. Future developments entail further analysis of the tested indicators. Moreover, an additional indicator, namely soil microarthropods, will be evaluated in the near future. Data were collected referred to the 2022-2023 agricultural campaign and are currently processed. These microarthropods have been demonstrated to respond sensitively to soil management practices and to correlate with beneficial soil functions.
- The assessment of the state of the art of alternative forms of organic methods led to a literature review on biodynamic agriculture. The reviewed scientific research indicated that under given production and pedo-climatic circumstances

the biodynamic method improves soil quality and biodiversity. However, further efforts are needed to implement knowledge regarding the socio-economic sustainability and food quality aspects of biodynamic products.

- The data recorded over the 30-year of the MoLTE trial showed that yields significantly decreased with time in both organic and conventional systems (about -79% and -37% for spring and winter crops, respectively). This decrease could be attributed to a substantial drop (about -40%) in cumulative rainfall during the vegetative crop cycle and an increase in temperature (+1°C). Organic winter crops constantly yielded about 21% less than the conventional ones while spring crops did not show significant differences. Despite the higher productivity by 21% in conventional winter crops, the organic system showed a considerably higher energy use efficiency *EUE*. For each unit of energy input, the energy output was found to be 33% higher in the organic system for winter crops. Even greater *EUE* was observed for spring crops, with a 44% higher efficiency in the organic. Therefore, the organic system undoubtedly exhibited better performance in terms of energy balance.

In conclusion, three are the challenges that Mediterranean agriculture needs to face 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 concept of "Perfect Storm" 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 of the three crises.

Backed by these considerations and by evidence of long term dynamics at the Montepaldi Long Term Experiment, I summarise the results of the present thesis as follows. Organic winter crops in internal hill land under semiarid conditions produce -21% per unit of land and +33% per unit of energy as compared to conventional farming. In a country like Italy that imports 2/3 of energy demand and cultivates only 12.5 million hectares of agricultural area used as compared to 21.9 millions in the '60, we can reasonably state that organic farming is an option to face the "Perfect Storm" in the Mediterranean. Spring crops showed a drastic decrease of productivity in the last 30 years both under organic and conventional farming in line with IPCC 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 in storing more water and plants in reaching deeper layers in the soil profile. Such aspects of organic farming are promising but apparently they are not sufficient in coping with water scarcity for spring crops. This calls for more advanced research on water stress resilient crop species and varieties appropriate for organic agriculture, as well as heterogeneous seed material having very diverse characteristics that allow it to evolve and adapt to variable growing conditions, including scenarios featured by severe water scarcity.

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