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Application of organic matter to alleviate soil sickness: effects on crop yield, soil properties and diseases suppression

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Preface

Within agricultural soil, non-living components and living organisms such as bacteria, fungi, protozoa, invertebrate animals and plant roots interact with each other contributing to the maintenance and productivity of agro-ecosystems. However, long-term use of intensive agricultural practices including monoculture, intensive tillage, intensive use of chemical fertilizers and application of agrochemicals negatively affect dynamics and functionality of agro-ecosystem, resulting in the "soil sickness" phenomenon. It represents a serious problem to the farmers because it causes a poor seed emergence, seedling mortality, stunted growth, reduction of yield and susceptibility to disease. Over the last 50 years, the researchers have largely studied soil sickness but the main cause has not yet been identified. However, several hypotheses have been proposed to explain it, including: i) soil nutrient depletion or imbalance; ii) build-up of soilborne pathogen and parasite populations coupled with a shift in soil microbial community composition and iii) release of phytotoxic and autotoxic compounds during decomposition of crop residues and plant litter. It was previously suggested that all proposed hypotheses have a common origin, i.e. the alteration of organic matter cycle caused by the intensive agricultural practices.

Starting from this consideration, the main objective of this thesis was to evaluate the impact that different organic management strategies (i.e., different organic amendment types and application frequencies) on the recovery of soil affected by soil sickness. In detail, the work is composed of five chapters. In "Chapter 1", an updated picture of the current knowledge on soil sickness, including a comparison between agroecosystems and natural plant ecosystems is provided. In "Chapter 2", an explorative study was conducted in order to understand the diffusion and the main factors involved in the soil sickness of intensive baby-leaf cultivation. Subsequently, a soil affected by soil sickness was conditioned with ordinary soil management (i.e. use of mineral fertilizers and fumigation) and different organic amendment treatments (i.e. different organic amendments and application frequency) in order to compare the effects on crop yield, quality and health, as well as on soil fertility and soil microbial communities at the end of the first (Chapter 3) and second (Chapter 4) experimental year. Finally, in "Chapter 5", soil conditioning for two years with ordinary and organic managements was carried out in order to evaluate the effects of these different treatments on disease suppression of soilborne phytopathogenic fungi and viruses.

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Chapter 1

SOIL SICKNESS AND NEGATIVE PLANT-SOIL FEEDBACK: A REAPPRAISAL OF HYPOTHESES

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Abstract

Soil sickness (SS) is the rise of negative conditions for plant vegetative and reproductive performances induced into the soil by the plant itself. In natural ecosystems, plant ecologists refer to SS as negative plant-soil feedback (NPSF). Scope of this review is to provide an updated picture of the current SS understanding by an explicit comparison between agro-ecosystems and natural plant communities. By an extensive analysis of literature, we found that SS is pervasive in agro-ecosystems, occurring in 111 cultivated plants belonging to 41 taxonomic families. Concerning NPSF in natural plant communities, we found evidence of this phenomenon for a total of 411 vascular plants belonging to 72 plant families. NPSF occur in most of the terrestrial ecosystems, including tropical and temperate forests, coastal sand dunes, old fields and grassland, deserts, as well as heathland and tundra. Three main hypotheses have been proposed to explain SS: i. soil nutrient depletion or imbalance; ii. build-up of soilborne pathogen and parasite populations, coupled with a shift in soil microbial community composition; iii. release of phytotoxic and autotoxic compounds during decomposition of crop residues. Evidences from both agro-ecosystems and natural plant communities undoubtedly ruled-out the nutrient deficiency as a primary causal factor. Moreover, the massive use of mineral fertilizers, especially under intensive cultivation systems, appears an incorrect strategy that only exacerbates the decline of soil quality by inducing acidification and salinization. Soilborne pathogens are often isolated from symptomatic plants and many autotoxic compounds have been identified and quantified from sick soil. However, both the pathogenic and autotoxicity hypotheses are still unable to fully explain the species-specificity, as well as the long durability of SS observed in field conditions. The recent discovery that extracellular DNA (exDNA) has self-inhibitory effects, support the autotoxicity hypothesis, nevertheless this is a totally new topic, and more solid and systematic field investigations are needed. A better understanding of the causes of SS is a necessary step to develop eco-friendly solutions to overcome this problem.

Keywords: Autotoxicity, Extracellular DNA, Plant residues phytotoxicity, Soil quality, Soilborne pathogens.

1. Introduction

Soil quality is one of the central factors that control yield and crop health in an agro-ecosystem (Larkin 2015). Soil quality is defined as the disposition of the soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health (Doran and Parkin, 1994). Agricultural practices like crop rotations, application of mineral fertilizers and organic amendment, tillage regime and the use of agrochemicals largely affect soil quality (Bastida et al., 2008; Parr et al., 1992; Wang et al., 2014). Indeed, soil quality derives from the interaction among physical, chemical and microbiological factors that, in turn, control water retention capacity, soil structure and stability of aggregates, organic matter dynamics, nutrient mineralization and suppression of soilborne pathogens (Abiven et al., 2009; Janvier et al., 2007).

In the last decades, the spread of intensive agriculture caused a significant decrease in the primary productivity worldwide, and this has been linked to soil deterioration (Bennett et al., 2012). Soil erosion, salinity, sodicity (Naidu et al., 1995), soil compaction (Drewry et al., 2008; Kukal et al., 2008), pollution by heavy metals and xenobiotics (Shen et al., 2005), decrease of soil organic carbon (Johnston, 1986), and the loss of beneficial microbiomes (Ibekwe et al., 2001), are all factors that reduce soil quality. In this broad context, a special case of soil quality decline is soil sickness.

Soil sickness (SS) is defined as the rise of negative conditions for plant vegetative and reproductive performances induced into the soil by the plant itself (Bennett et al., 2012; Huang et al., 2013; Mazzoleni et al., 2007). This phenomenon is known in agronomy as "soil fatigue" (Schreiner and Sullivan, 1908), or "replant disease problem" (Mai and Abawi, 1978). SS has been demonstrated to be strongly species-specific, i.e. mainly affecting individuals of the same species. In particular, sensitivity to SS decline with the increase of the phylogenetic distance among species (Zucconi 2003). Moreover, in the last three decades, researchers recognized the importance of SS in shaping the structure of natural plant communities and for the maintenance of their species diversity (Van der Putten et al., 2013). Plant ecologists refer to SS as Negative Plant-Soil Feedback (NPSF), stressing the mutual, although negative interactions between plant and soil. In ecology, NPSF is often referred as 'Soil Carry-over Effects' (Bartelt-Ryser et al., 2005), 'Legacy Effects', or 'Historical Contingencies' (Kardol et al., 2007). However, despite the decadal research efforts addressed to SS and NPSF topics, the underlying causative mechanisms are still poorly understood, yet highly debated.

The general scope of this review is to provide an updated picture of the current SS understanding. After an historical overview, a comprehensive analysis that includes studies on SS and NPSF has been discussed, thus providing a complete description of the mechanisms behind this complex

phenomenon. Moreover, an explicit comparison between agro-ecosystems and natural plant communities has been likewise included in order to promote new approaches to the understanding of this phenomenon. The review specifically aims to:

i. Assess the occurrence of SS and NPSF in agro-ecosystem and natural plant communities;
ii. Explore the underlying causative mechanisms, by comparing the main hypotheses proposed to explain this phenomenon;
iii. Describe the methodological approaches, highlighting their strength and weakness.

2. Historical overview

During the Holocene, the human beings changed their lifestyle, switching from nomadic (i.e. moving from one place to another in response to variations in the season and climate) to sedentary (i.e. living for a prolonged period in the same place) (Gupta, 2004). Thus, animal hunting and natural fruit gathering were replaced by sedentary agriculture, with a consequent impact on the interactions between plant and soil.

The first evidence of soil sickness dates back to the time of ancient Greeks and Romans. In his botanical works, Theophrastus (ca. 371 - 287 BC) reported that chickpea (*Cicer arietinum*) doesn't reinvigorate the soil in which dwells but "exhaust" it (Rice, 1984). In the Roman Empire, Columella (4 – 70 AD) in his epic poem *De Rerum Rusticarum*, pointed out that the practice of planting a single crop year after year on the same land, such as barley, leads to SS. To overcome this problem, he suggested the use of manure or other organic materials as soil amendment, or the practice of crop rotation and fallow instead of monoculture. In the same period, Pliny the Elder (23-79 AD) wrote in his encyclopedic work "*Natural History*" that the plants grown near and below the shade of black walnut (*Juglans nigra*) are damaged by its own residues. To explain this phenomenon, later known as *allelopathy*, he hypothesized that plant roots or leaf litter could release phytotoxic substances in the surrounding environment which affect the growth of other plants (Rice, 1984).

In the following centuries agronomists, horticulturists and foresters investigated the phenomenon of SS, but only in the 20th century a significant increase of scientific knowledge was achieved thanks to the development of new analytical techniques and scientific instruments. Pioneering works in the early 1900s reported evidences that phytotoxic compounds are involved in SS (Benedict, 1941; Proebsting and Gilmore, 1941; Russell and Petherbridge, 1912; Schreiner and Shorey, 1909). The attention on phytotoxic compounds derived from root exudates, decaying plant debris and sick soil reached the peak in the 1960s and 1970s (Börner, 1960; Collina and Zucconi, 1967; Patrick, 1971).

The role of phytotoxic compounds in SS was recently challenged by the discovery that extracellular DNA had species-specific inhibitory effect on plants (Mazzoleni et al., 2015a).

In the 1960s, several studies demonstrated that soil sterilization reduces or avoid the rise of SS, suggesting that detrimental microbes are involved (Hoestra, 1968; Savory, 1966). Since that time, a large number of soilborne pathogens and plant parasitic nematodes have been isolated from soils or diseased plant roots. Patrick and co-workers (1963) firstly proposed the hypothesis that phytotoxic compounds, either released by roots during exudation or during decomposing of plant residues, indirectly promote the activity of soilborne pathogens by weakening the plant and, consequently, reducing its resistance.

Plant ecologists, in contrast, did not recognise the importance of SS in shaping natural ecosystem until the 1990s. SS probably was unnoticed because in natural ecosystems plants live in mixed communities, with monospecific stand occurring only under specific ecological conditions (Mazzoleni et al., 2007). Because the impact of SS on conspecifics is the reduction of individual's vigour and dominance in favour of other species, in mixed communities it is difficult to observe and isolate SS effects. This can be detected only in long-term field studies that monitor population dynamics at an individual scale. In this regard, Watt (1947) for the first time described species alternation in time and space in heathland dominated by the shrub *Calluna vulgaris*. Here, this species was unable to regenerate over the same physical place, driving to a sort of a natural rotation with other species. Few years later, in USA and Australia it was noticed that after clear-cutting several tree species, including *Sequoia sempervirens* (Florence, 1965), *Eucalyptus pilularis* (Florence and Crocker, 1962), and *Grevillea robusta* (Webb et al., 1967), were unable to regenerate in stand previously occupied by conspecifics. The authors proposed that regeneration failure was caused by the accumulation of unidentified, autotoxic factors (Webb et al., 1967). However, these scattered observations were considered by ecologists as a "noise" and the SS process was ignored as important in ecological frameworks. In the 1990s, thanks to some key studies, SS gained consideration to fully understand natural plant communities. Van der Putten et al. (1993) demonstrated that soilborne pathogens and plant-parasitic nematodes are responsible for the successional replacement of Marram grass (*Ammophila arenaria*) in sand dune communities. Bever (1994) reported that herbaceous species from old-field suffer when cultivated in soil previously used by conspecific, and coined the term "negative plant-soil feedback" (NPSF). Later, the same author proposed a conceptual framework where NPSF process was mechanistically linked to species coexistence, successional dynamics and the maintenance of plant diversity (Bever et al., 1997). Since then, the research interest on NPSF rose exponentially driving to a burst of publications on this topic (Fig. 1).

3. Occurrence of soil sickness and negative plant-soil feedback

SS is pervasive in agro-ecosystems, and Table 1 provides an updated list of cultivated plants susceptible to SS. We found scientific evidence for the occurrences of SS in 111 species, belonging to 41 taxonomic families. In addition, SS has been observed by farmers in many other cultivated plants, but these data are mostly unpublished. SS is a severe problem for field crop cultivations, in horticulture and floriculture as well as for orchard trees (Table 1). In fact, it is very difficult to find any herbaceous plantations or orchards that do not experience the consequences of SS when cultivated in monoculture and monosuccession. As Table 1 highlights, major crops and trees are affected by SS including wheat, corn, rice, sugarcane, alfalfa, soybean, grape, and, among trees, peach, apple, olive, citrus, tea and coffee. The length of plant life cycle correlates with the susceptibility to SS, with short-living plants (annuals) being the most sensitive, followed by perennial forb, tree, vine, shrub and perennial grass (Fig. 2b). Interestingly, in 21.6% of the cases, SS was found for plants belonging to the *Poaceae* and *Fabaceae* families (Fig. 2a). Members of the *Fabaceae* are especially sensitive to SS, with many species severely affected (e.g. alfalfa, cicer, clover, soybean, bean, etc.; Table 1). SS has been also reported for other plant families, including *Apiaceae*, *Asteraceae*, *Brassicaceae*, *Cucurbitaceae*, *Liliaceae, Rosaceae* and *Solanaceae* (Table 1). Another evidence that SS is a global phenomenon is that its cases have been observed and described for agro-ecosystems in various regions of the world, characterized by different climatic conditions and soil types (Fig. 2c). Examples of less common, yet recurring, SS cases in tropical and sub-tropical environments include *Coffea arabica*, *Musa* sp., *Oryza sativa* and *Saccharum officinarum* (Table 1).

Concerning NPSF in natural plant communities, from an extensive review of the literature we found evidence of this phenomenon for a total of 411 vascular plants belonging to 72 plant families (Annex table 1; Fig. 3a). NPSF has been reported in most of the terrestrial ecosystems, including tropical and temperate forests (Bennett et al., 2017; Mangan et al., 2010), coastal sand dunes (Oremus and Otten, 1981), old fields and grassland (Bezemer et al., 2006; Olff et al., 2000), deserts (Rutten et al., 2016), salt marshes (Castellanos et al., 1994), as well as heathland and tundra (Bonanomi et al. 2005a). In our data set, NPSF occurrences were higher in temperate grassland followed by temperate forest, arid ecosystems and tropical forest (Fig. 3c). Few cases have been reported from wetlands and shrubland (Fig. 3c). These data, however, are partially influenced by the American and North European works, as they carried out most of the studies reported so far. Moreover, grasslands has been often selected as model system because plants are small, short-lived and easy to manipulate compared to shrubland and forest ecosystems. Therefore, the lower occurrence of NPSF studies in

ecosystems such as the Mediterranean shrublands or the boreal forest, does not necessarily indicate that these ecosystems are less subjected to the NPSF.

NPSF affects plants of different life forms including annual (Kardol et al., 2007), perennial herbs and sedge (Jordan et al., 2008), shrubs (Sigüenza et al., 2006), and trees (Reinhart et al., 2005). However, annual plants occur more frequently than shrubs and trees, with some cases reported for vine and sedge (Fig. 3b; Annex table 1). This observation is consistent with the meta-analysis by Kulmatiski et al. (2008), reporting that annual and biennial plants experience more intense NPSF than perennials, and particularly woody species. The higher susceptibility of annual species toward negative feedback can be explained with the absence of storage organs (e.g. rhizomes, tubers, bulbs, twigs), which make these plants less capable of facing environmental stresses (Pastor and Durkee Walker, 2006). By the taxonomic point of view, *Poaceae*, *Asteraceae* and *Fabaceae* are the families with the highest number of reported cases of NPSF (Annex table 1). In natural ecosystems, nitrogen-fixing *Fabaceae* are a key step in successions where they play an important role in the accumulation of nitrogen stocks into the soil (Bellingham et al., 2001; Walker et al., 2003). However, these species -after a peak of dominance that lasts few years- rapidly disappear (Chapin et al., 1994; Stinca et al., 2015). This can be explained by NPSF that exacerbates the competitive interaction with later successional species. For example, Teste et al. (2017) reported that nitrogen-fixing species from Mediterranean shrublands of Southwest Australia suffer a stronger negative feedback compared to other woody species in the same environment. The high susceptibility of *Fabaceae* to NPSF is consistent with evidences from agro-ecosystems, where plants belonging to this family suffer intense and long-lasting SS (Table 1).

In agro-ecosystems, where plants are cultivated in even-aged and pure monoculture stands, SS is easily detected as poor seed emergence or poorly developed patches (Fig. 4a). For perennial plants and orchard trees, a common but generic symptom of SS is the stunted growth and reduction of yield. In natural ecosystems, instead, plants generally grow in mixed communities and, therefore, the impact of NPSF depends on the growth form. For instance, a number of empirical and modelling studies demonstrated that the acquisition of different propagation modes provide a way to escape NPSF that develops in the "home" soil (Bever, 1994). Thus, different spatial patterns emerge from the interaction between NPSF and growth forms with different life spans, as it occurs for annual herbs and trees (Vincenot et al., 2017). For trees and shrubs NPSF is spatially localized under their canopy, and so these plants escape the detrimental effects of "home soil" via seed dispersal (Packer and Clay, 2000). In other words, plants with a single rooting point, exhibit a distance-dependent inhibition, a sort of seedling repellence from their mother plant (Fig. 4b). Seedling establishment is reduced or even

completely absent under the canopy of the mother plant due to NPSF despite the high abundance of seeds, thus producing the so-called Janzen-Connell recruitment distribution (Fig. 4b; Janzen, 1970). This distribution has been described for shrubs (Bonanomi et al., 2008; Lambers and Clark, 2003) and, most commonly, for trees in both temperate and tropical forests (Augspurger, 1984; Mangan et al., 2010; Packer and Clay, 2000). At a community scale, the Janzen-Connell recruitment distribution drives to the alternation in time and space of tree species in forest ecosystems (Fox, 1977; Whittaker and Levin, 1977), thus contributing to the maintenance of species diversity (Mangan et al., 2010).

Perennial herbaceous plants capable of clonal propagation, can actively move away from the hostile "home soil" by selective proliferation of new ramets in suitable soil patches (Blundell and Peart, 2004; Olff et al., 2000). This type of clonal spreading is characterized by wave-like patterns, and by the production of regularly shaped rings. In a recent review, Bonanomi et al. (2014) reported that herbs, shrubs and trees capable of clonal propagation, during their ontogenetic cycles, produce clones with a "ring" shape that progressively degenerate in the older inner area, thus producing a "dieback" central zone (Fig. 4c). This vegetation pattern has been also called fairy rings, rings, hollow crowns, central dieback, and monk's tonsure-like gaps (Adachi et al., 1996; Lewis et al., 2001; Watt, 1947). Interestingly, in many cases the inner area is colonized by multiple species, different from the dominant plant that generated the ring, thus resulting in an increased local biodiversity (Castellanos et al., 1994; Bonanomi et al. 2005b). Finally, short-lived plants (i.e. annual and biennial) can avoid the "home soil" by random searching for NPSF free sites through seed dispersal. In this regard, some studies reported that in natural grasslands short-lived plants show a rapid and continuous turnover at small spatial scales that, at the same time, results in a stable plant assemblage at community scale (Vincenot et al., 2017). In other words, plants of different species alternatively occupy soil patches in time and space, resulting in a rapid rotation of species. This spatial-temporal pattern has been called "Carousel model" (Maarel and Sykes, 1993) because plants continuously move in time and space, changing their spatial position into the grassland. In this contest, a parallelism between natural ecosystems and agro-ecosystems can be highlighted: in the first, plants move away from sick "home soil" through seed dispersal, or clonal propagation resulting in a self-emerging species alternation or rotation. In agro-ecosystems, instead, farmers overcome SS through the ancient, yet very effective, agronomic practice of crop rotation.

4. Mechanisms behind soil sickness and negative plant-soil feedback

Soil sickness is a complex, multi-factorial phenomenon influenced by plant species, crop rotation and soil management practices. In addition, environmental factors such as climate and soil type may

increase the complexity of the phenomenon (Venugopalan and Pundarikakshudu, 1999). In order to explain the mechanisms causing SS and NPSF, three main hypotheses have been proposed:

4.1. soil nutrient depletion or imbalance (Howeler, 1991; Xiang et al., 2009);

4.2. build-up of soilborne pathogen and parasite populations (Manici et al., 2013; Packer and Clay, 2000), coupled with a shift in soil microbial community composition (Kardol et al., 2007; Klironomos, 2002);

4.3. release of phytotoxic and autotoxic compounds during decomposition of crop residues (Singh et al., 1999; van de Voorde et al., 2012), or plant litter (Mazzoleni et al., 2015a).

4.1. Soil nutrient depletion or imbalance

The first hypothesis proposed to explain SS and the consequent decline of crop production, suggests that depletion or immobilization of soil nutrients cause deficiency in plants (Börner, 1960; Ehrenfeld et al., 2005). This hypothesis invokes the depletion of below-ground nutrients in the soil previously occupied by conspecifics. The majority of evidences from agro-ecosystem and natural plant communities does not support the nutrient depletion hypothesis.

At the beginning of the 1900s, pioneering studies compared ashes and nutrient content from different plants (reviewed by Börner, 1960). Nevertheless, differences in mineral composition resulted unrelated to SS and unable to explain the species-specificity of the phenomenon. A number of subsequent agronomical studies evaluated the capability of nutrient fertilization to overcome SS, but most of the experiments demonstrated that mineral fertilizers did not restore the normal growth in sick soils (reviewed by Zucconi, 2003). For example, in their study about the effects of cucumber monocropping on soil quality and plant growth performance, Zhou and Wu (2015) found that the content of macronutrients, such as nitrogen, phosphorus and potassium, in the soil increases with the number of cropping cycles. However, SS increased over time in monocropping, and the effects were particularly dramatic after five production cycles.

Further evidences against the nutrient depletion hypothesis came from soilless cultivation experiments. Many experiments conducted in hydroponic systems, where the level of nutrients was continuously adjusted and balanced in function of the vegetative stage of the crop, showed that the reduction of plant performance observed with the "old" solutions cannot be related to a deficiency of any nutrients. In these cases, researchers ascribed SS to toxic substances released by the root system (Asaduzzaman and Asao, 2012; Asao et al., 2007; Yu et al., 1993), the spread of pathogenic microbes (Vallance et al., 2009), and to the interaction between toxic substances and harmful microorganisms (Ye et al., 2004; Zhang, 1993).

Another set of data against the nutrient depletion hypothesis come from the observation that legumes, nitrogen-fixing species, develop severe NPSF (Tables 1 and Annex table 1; Figs 2 and 3). This is quite unexpected, considering that such type of plants enriches the soil with nitrogen and phosphorus. For instance, seedlings of *Medicago marina*, a plant that colonizes sandy shore in the Mediterranean basin, suffer a strong self-repulsion from mother plants (Bonanomi et al., 2008). Greenhouse experiments with *M. marina* showed that seedlings are strongly inhibited in the "home" soil collected under the crown of the mother plant, in comparison to seedlings grown in the same type of soil not affected by conspecific, and taken from the adjacent sandy beach deprived of nutrients. Surprisingly, the seedlings were stunted in "home" soil despite having 5 and 6 times the amount of nitrogen and phosphorus and a lower salinity compared with the surrounding sandy soil (Bonanomi et al., 2008). Similar findings were reported by Jennings and Nelson (2002) for the congeneric *Medicago sativa* in agricultural fields. More recently, Stinca et al. (2015) reported that the legume shrub *Genista aetnensis* that colonize the bare lava flow of the Vesuvius Grand Cone ameliorates soil characteristics. In detail, *G. aetnensis* in a relatively short time span (i.e. ~40 years) is able to build-up an island of fertility under its canopy by accumulating stock of organic carbon, nitrogen, phosphorus, potassium, calcium, and magnesium and by improving the hydrological properties of the soil. On the other hand, *G. aetnensis* seedlings were absent in the field under the canopy of conspecifics, and greenhouse bioassays showed that seedlings growth was inhibited in "home" soil compared to the barren, nutrient deprived substrate collected far from the canopy of conspecifics. Noteworthy, coexisting phylogenetically-unrelated plants thrive in the soil enriched with nutrients by *G. aetnensis* (Stinca et al., 2015). Similar self-inhibitory effects have been demonstrated for other nitrogen-fixing species including *Alnus sinuata* during colonization of glacier moraine in Alaska (Chapin et al., 1994), *Acacia papyrocarpa* in the Australian desert (Facelli and Brock, 2000), and for the nitrogen-fixing tree *Hippophae rhamnoides* in sandy shores of North Europe (Oremus and Otten, 1981). All these cases show the formation of "islands of fertility" with inhibitory effects on conspecific younger individuals.

A further evidence against the nutrient depletion hypothesis came from clonal perennial plants forming "ring" (Fig. 4c). For this type of plants, several studies reported a higher nutrient concentration in the inner "dieback" area compared with the soil outside the ring (Adachi et al., 1996; Castellanos et al., 1994; Incerti et al., 2013; Lewis et al., 2001; Otfinowski, 2008; Ravi et al., 2008; Wikberg et al., 2002). In an early study, Curtis and Cottam (1950) found that the prairie sunflower *Helianthus rigidus* in the field is able to form clones with central "dieback". In a subsequent experiment, the same authors reported that the growth of *H. rigidus* is not improved after the

application of mineral fertilizer in the inner area. Similarly, Lanta et al. (2008) observed that the soil in the dieback area of the sedge *Eriophorum angustifolium*, showed a significantly higher nutrient content compared to the external soil, and the same pattern has been reported for the perennial grasses *Brachypodium rupestre* (Bonanomi and Allegrezza, 2004), and *Bromus inermis* (Otfinowski el al., 2016). Moreover, some studies reported an increased water holding capacity in the "dieback" zone of the clones, a result associated with the higher soil organic matter (Lanta et al., 2004; Pemadasa, 1981; Pignatti, 1997). These results indicate that self-inhibition paradoxically occur in "home" soil where a higher soil quality is usually recorded.

In conclusion, evidence from both agricultural and natural ecosystems indicate that the nutrient depletion hypothesis cannot be a satisfactory explanation for the development of SS and NPSF. However, the phenomenon of soil nutrient depletion does exist and has been frequently observed in poor and undeveloped countries where the use of fertilizers, both organic and synthetic, often represents a limit due to their poor availability or high cost. For example, in a study on yield decline in banana (*Musa* sp.), Bekunda (1999) shows that the intensification of intercropping practice, the removal of crop residues and the poor application of fertilizers have led to a loss of soil fertility and, consequently, to a reduction in banana production. Similar results were reported for the cultivation of cassava (*Manihot esculenta*) by Howeler (1991). In this case, the continuous cultivation under low input of fertilizers has determined a soil nutrient depletion, especially in potassium, with negative effects on crop production.

4.2. Soilborne pathogens and microbial shift

Soil microorganisms are key components of natural and agricultural ecosystems, given their contribution to chemical and biological processes including break-down of organic matter, carbon and nitrogen cycles, stabilization of soil aggregates, nutrient acquisition, and degradation of environmental pollutants (Bever, 1994; Bronick and Lal, 2005; Ehrenfeld et al., 2005; Kardol et al., 2006; Reinhart and Callaway, 2006). The composition and abundance of soil microbes are controlled by soil properties (e.g., temperature, moisture, aeration, pH), but also by higher plants through rhizodeposition (Paterson et al., 2007), and accumulation of leaf and root debris (Wardle et al., 2004; Zak et al., 2003). In this way, plants promote the development of beneficial microbes such as nitrogen fixing bacteria and mycorrhizal fungi (Artursson et al., 2006; Hayat et al., 2010), but may also favour the spread of soilborne pathogens, plant parasitic nematodes and deleterious rhizobacteria (Bennett et al., 2012; Huang et al., 2013; Shipton, 1977).

The hypothesis that SS is due to the accumulation of pathogens in the soil was proposed after the observation that soil sterilization restores crop productivity in soils subjected to monoculture (Savory, 1966). For example, Hoestra (1965) reported that the poor growth of young cherry trees (*Prunus avium*) planted on soil previously occupied by the same species ("home" soil), was associated with a strong infestation by the endoparasitic nematode *Pratylenchus penetrans*, and by the soilborne fungus *Thielaviopsis basicola*. When the soil was fumigated, instead, an improved growth was observed supporting the hypothesis that harmful microorganisms are the main cause of the replanting problem. Since then, the efficacy of soil sterilization in restoring sick soil has been proved in several agro-ecosystems (Table 1). Pankhurst et al. (2005) suggest that the poor growth and yield decline of sugarcane (*Saccharum* spp.) grown in continuous monoculture is due to the presence of deleterious soil organisms. In particular, they reported that both soil fumigation and the application of fungicide combined with nematicide increased the growth and yield of sugarcane in comparison with the untreated soil. Concerning orchards, replant disease of apple has been reported in all major apple growing regions and extensively studied (Mazzola and Manici, 2012). Mazzola (1998) assessed the relative role of different soil microbial groups in the development of apple replant disease by the application of selective pesticides. The results demonstrate that the application of fungicides was as effective as soil pasteurization in improving the growth of plants, whereas the application of antibiotic and nematicide did not improve plant performances. *Cylindrocarpon destructans*, *Pythium* spp., *Phytophthora cactorum*, and *Rhizoctonia solani* were repeatedly isolated from symptomatic plants in field conditions, confirming the key role of fungi and oomycetes in apple replant disease. However, the relative occurrence of *Pythium* and *R. solani* isolates within the root rot microbial complex largely varied from site to site. In Italy, Manici et al. (2003) confirmed that apple replant problem is associated with a complex pathogenic microbiota that includes *R. solani*, *P. intermedium*, *Cylindrocarpon* spp. and *Fusarium solani*. Several pathogens were involved also in the black root rot of strawberry, where *R. solani*, *C. destructans*, *F. oxysporum*, and *F. solani* play the major role (Manici et al., 2005; Neri et al., 1998). In natural ecosystems, Packer and Clay (2000) provide clear evidence of NPSF driving to the Janzen-Connell recruitment pattern for black cherry (*Prunus serotina*) in temperate forest of USA. In detail, the authors observed extensive seedling failure under conspecific adults, by identifying *Pythium* sp. as the primary causal agent. It is notable, from the aforementioned studies, that most of the pathogens associated with SS as well as NPSF are polyphagous fungi and oomycetes. The evidence that soilborne pathogens are consistently isolated from symptomatic plants supports the pathogenic hypothesis, but the polyphagous nature of these pathogens does not fit the paradigm because SS is highly species-specific. In fact, SS has been

associated with species-specific pathogens only in very few cases (Table 1). An exception is the asparagus (*Asparagus officinalis*) replant early decline caused by *F. oxysporum* f. sp. *asparagi* (Blok and Bollen, 1996).

Although extensive research on soilborne pathogens and parasites has been carried out in agricultural systems, similar studies in natural eco-systems are relatively rare. On the other hand, the studies about NPSF in natural plant communities are numerous (Fig. 1; Annex table 1), but most of these used a multi-spet, "black-box" approach (Bever, 1994; Kardol et al., 2007; Klironomos, 2002). In the first step, defined as the conditioning phase, soil is cultivated with selected plant species for which the feedback mechanism is investigated. During this phase, plant interacts with biotic and abiotic soil components by altering them. In the second step, the effects of the conditioning phase are assessed by comparing the growth of a new plant in self-cultivated or "home" soil, and non-self-cultivated soil also indicated as "away" soil. If the plant grows more in the self than in the other cultivated soils, the feedback is considered positive, otherwise is negative. A large and still growing body of data demonstrates that the negative feedback is more common than the positive one (Annex table 1; Kulmatiski et al., 2008). Moreover, several studies found significant changes in microbiota composition using culture-based as well as culture-independent methods (Van Der Heijden et al., 2008; Bever et al., 2013). In some experiments, NPSF was transferred from different soils by using small aliquots of "sick" soil as a microbial inoculum (Kardol et al., 2007). Many researchers interpreted the observed NPSF as a result of some, often undescribed, microbial shift that occurs during the conditional phase of the experiment. In accordance with this, we pointed out that in literature the microbial shift was find as the main putative cause of NPSF in 65.8% of the studies (Annex table 1; Fig. 4). However, for a better understanding of the role of soil biota in NPSF, the evaluation of composition and changes in the entire microbial community is a necessary step. Recent studies have demonstrated that the net effect of plant-soil feedback is the balance between beneficial and detrimental microbes. Bennett et al. (2017), using 55 populations of North American trees reported that soil collected beneath conspecifics, showed NPSF for most of the studied species. Most notably, the type of mycorrhizal association with plant species explained a large fraction of the variation in NPSF, with arbuscular mycorrhizal trees suffering a more intense NPSF than ectomycorrhizal ones. The authors suggested that ectomycorrhizal trees may protect plant roots from soilborne pathogens that accumulate under conspecifics. Similar findings were reported by Teste et al. (2017) from hyper-diverse Australian shrublands. In this work NPSF has been considered as the result of an imbalance of soil microbiota, with plants harboring ectomycorrhizal fungi that are more protected from detrimental microbes compared to plants that establish the symbiosis with arbuscular

mycorrhizal fungi. It is interesting to note that many cultivated plants, which suffer strong SS, are associated with arbuscular mycorrhizal fungi.

The hypothesis that SS is associated to soilborne pathogens presents some strengths and several weaknesses. The effectiveness of soil sterilization to overcome SS is often interpreted as a clear-cut proof that harmful microorganisms are the main driving factors of SS. However, an improved plant growth in sterilized compared to non-sterilized soils can be related also to other side effects of this treatment. Soil sterilization alters biotic and abiotic soil properties, providing a nutrient flush resulting from a rapid mineralization of the dead microbes (Troelstra et al., 2001). In addition, organic phytotoxic compounds may be subjected to thermal degradation. Therefore, the greatest availability of nutrients or the degradation of toxic compounds induced by soil sterilization may accidentally reduce the negative effects and promote positive vegetative responses in plants driving to ambiguous interpretation of the results (Troelstra et al., 2001). On the other hand, the frequent isolation of pathogenic oomycetes, fungi and parasitic nematodes from symptomatic plants strongly supports the pathogenic hypothesis. However, the observation that most of the isolated microbes are polyphagous is not coherent with the species-specificity of SS.

In the last few years, the assumption that sick soil is associated to one or few specific microbes progressively evolved towards a more complex idea that involves an unbalance in the microbiota that generates inhospitable soil conditions. Recent studies based on high-throughput sequencing of bacterial and eukaryotic rRNA gene markers revealed that soil is inhabited by thousands of different species that form complex food-web (Mendes et al., 2013; Bonanomi et al., 2016). The extensive application of new analytical tools will be very useful to establish if a sick soil is related to an overall shift in soil microbiota structure, rather than to changes in single or few microbial species.

4.3. Phytotoxicity and autotoxicity

The idea that harmful chemical compounds, either released through root exudation or by decaying of plant debris, are involved in SS dates back at the beginning of the 1900s (Russell and Petherbridge, 1912; Schreiner and Shorey, 1909). Only few studies, however, clearly demonstrated that plants exudate trough the roots chemical compounds that specifically harm conspecifics (Perry et al., 2005; Webb et al., 1967). The authors explain this event as a density-dependent regulation of population to avoid overcrowding and reduce intraspecific competition. The idea that SS could be caused by actively released toxins has been heavily criticized because such compounds are rapidly degraded by the soil microbes into non-toxic molecules, thus having a limited impact in field conditions (Fitter, 2003; Harper, 1977).

On the other hand, many studies reported that plant residues, either leaf or root debris, can have an inhibitory effect on plant growth (Table 1; Fig. 5). In controlled conditions, soil amendment with crop residues derived from conspecific impaired root and shoot growth of peach (Collina and Zucconi, 1967; Proebsting and Gilmore, 1941; Tagliavini and Marangoni, 1992), apple (Borner, 1959), olive (Endeshaw et al., 2015), tea, coffee (Putnam, 1994), alfalfa (Miller 1996), and many herbaceous plant species (review by Patrick, 1971; Putnam, 1994; Table 1). Data from natural ecosystems further demonstrated that leaf litter can have a detrimental impact on plant growth. Three recent studies, based on 21 (Lopez-Iglesias et al., 2014), 64 (Bonanomi et al., 2011c) and 65 (Meiners, 2014) different plant residues, demonstrated that litter inhibitory effects are common, but may largely vary in relation to the composition of plant residues, which in turn depends on plant biochemical activity and on litter decomposition stage. Bonanomi et al. (2006) reported that phytotoxicity of leaf and root debris depends on plant functional type with the following rank: annuals >> perennials ≥ woody. Noteworthy, undecomposed leaves of nitrogen-fixing species were invariably the most toxic plant tissue.

Microbial decomposition plays a key role in affecting phytotoxicity of plant debris. A better understanding of this process is crucial to appreciate the real role of crop residues and plant litter on SS and NPSF. During decomposition, the abundance and activity of nitrogen and phytotoxic compounds continuously change over time, because of sorption and polymerization on soil organic matter and clay minerals, as well as the chemical transformation driven by soil microbes (Blum et al., 1999). Considering these processes, two mutually non-exclusive hypotheses have been proposed to explain the inhibitory effect of plant debris on root growth: nitrogen (N) immobilization by microbial competition (Hodge 2004), and phytotoxicity by labile, low molecular weight organic compounds (Rice, 1984). According to the first hypothesis, in presence of decaying plant residues with a high C/N ratio, saprophytic microbes would compete with plants for N, causing a temporary immobilization of this nutrient (Hodge et al., 2000; Fig. 5). The second hypothesis sustains a direct negative effect on root growth exerted by a wide array of inhibitory compounds, early released by decomposing litter, including short-chain organic acids (Armstrong and Armstrong, 2001; Huang et al., 2010), tannins (Mizutani et al., 1979) and phenols (Chen et al., 2005; Chon et al., 2002). Examples of toxic compounds involved in SS and isolated from soil and plant debris include phlorizin for apple (Borner, 1959), amygdalin for peach (Patrick and Koch, 1958), medicarpin for alfalfa (Miller, 1996), caffeine for coffee (Chou and Waller, 1980), and coumaric, syringic and vanillic acids for rice (Chou and Lin, 1976). In this context, microbial decomposition is of utmost importance because it affects the impact of plant residues on plant growth, by modulating the relative abundance and activity of

phytotoxic compounds. Studies that specifically investigate the role of microbial decomposition reported a rapid degradation of most allelochemicals into non-toxic molecules in the early phases of this process (An et al., 2001; Bonanomi et al., 2011c). These studies demonstrated the occurrence of a temporal phytotoxic 'window' during decomposition of crop residues, which ranged from 5 to 30 days. In all cases, however, phytotoxicity dropped in time and, after 60-90 days of decomposition, "aged" organic matter showed neutral or even stimulatory effects on root proliferation.

All the aforementioned data, related to natural as well as to agro-ecosystems, demonstrated that root growth inhibition by undecomposed plant residues is a general phenomenon not restricted to few "allelopathic" plants. However, it is also well-established that phytotoxicity of plant debris is a transient phenomenon that usually lasts from few days to some weeks. Thus, some researchers raised serious concerns about the possible role of plant residue phytotoxicity on SS and NPSF (Fitter, 2003; Van Der Putten, 1997). Two main criticisms were addressed: i. toxins from plant debris are rapidly degraded by soil microbial activity, being ineffective after a few weeks, while SS in the field can last for months or even years; ii. many, if not all, of the organic compounds extracted from sick soil and plant residues (e.g. Huang et al., 2013; Rice, 1984; Singh et al., 1999; Chen et al. 2015), showed a general phytotoxicity, which is in contrast with the species-specificity of SS. For example, Armstrong and Armstrong (2001) associated *Phragmites australis* "die-back" to the accumulation in the sediment of propionic, butyric and caproic acids and sulphides produced during anaerobic decomposition of root and rhizomes. However, these low-weight carboxylic acids showed phytotoxic effects on a wide range of higher plants (Himanen et al., 2012). Singh et al. (1999) reported 76 cases of plant autotoxicity caused by their own residues or root exudates. In Table 1 we find out up to 60.2% of the experimental studies reporting soil sickness in agro-ecosystems ascribed to autotoxicity. However, as observed also by Singh et al. (1999), autotoxicity was demonstrated exclusively using conspecific as test plants, while other phylogenetically unrelated species were not considered. This is a common bias in the current literature that leaves serious doubts about the real species-specificity of plant residues autotoxicity.

It appears clear that most of the low molecular weight phytotoxic compounds can hardly explain SS and NPSF because of their short persistence into the soil and lack of species-specific effects. A recent finding, reporting the species-specific inhibitory effects of extracellular DNA (exDNA) (Mazzoleni et al., 2015a), may reconcile the toxicity hypothesis with the occurrence of SS. Mazzoleni et al. (2015a,b) reported that fragmented extracellular self-DNA (i.e. DNA originating from conspecifics) produces species-specific inhibitory effects on several wild plants. First, the analysis of plant debris showed a significant accumulation of exDNA during the decomposition process,

although in fragmented forms. Thereafter, *in vitro* experiments demonstrated that the inhibitory effect of self-DNA was highly species-specific. Noteworthy, the authors found that only highly fragmented self-DNA was effective (i.e. fragment size range between 50 and 2000 bp). In addition, the application of activated carbon, known to selectively adsorb allelopathic organic compounds, was not able to remove autotoxicity, neither to restore plant growth. Conversely, heterologous exDNA from phylogenetically unrelated plant species had no inhibitory effects. This is consistent with a previous work (Paungfoo-Lonhienne et al., 2010), where heterologous DNA was taken-up by root and used as a source of phosphorus. Moreover, the strong persistence of exDNA in the environment (Levy-Booth et al., 2007) is in agreement with the persistence of SS observed in both natural vegetation and agro-ecosystems. Finally, since exDNA is destroyed during soil sterilization by autoclaving or gamma irradiation, the well-known effectiveness of this treatment to overcome SS cannot be used to discriminate between exDNA toxicity and the pathogenic hypotheses. Thus far, the authors concluded that self- exDNA is a good putative candidate to explain SS and NPSF (Mazzoleni et al., 2015a). The hypothesis that exDNA can be involved in SS is intriguing, but further works are needed to validate this idea. In detail, quantitative data are required about the accumulation of self- exDNA in field conditions, as well specific experiments to confirm the inhibitory effect of purified conspecific DNA on seed germination and root growth of cultivated plants (Barbero et al., 2016).

Besides the underlying causal mechanisms, the autotoxicity hypothesis poses an evolutionary paradox: why a plant species should harm its own off-springs? Some authors suggested that autotoxicity acts as a density-dependent regulation mechanism to avoid population overcrowding (McNaughton, 1968; Perry et al., 2005; Singh et al., 1999). Our idea, instead, is that all living organisms, such as bacteria, fungi, algae and animals, produce by their metabolic pathways different catabolites (by-products and wastes). Why higher plants should be an exception? Interestingly, catabolic wastes that are toxic for the producing species may be at the same time a resource for other species. In this regard, it is notable that floating plant (e.g. *Eichhornia crassipes*, *Lemna* spp., *Pistia* spp.), mangrove forests (*Avicennia* spp., *Nypa* fruticans Wurmb., *Rhizophora* spp.), seagrass (*Posidonia* spp., *Thalassia* spp., *Zostera* spp.), seaweed and kelp forests (*Fucus* spp., *Laminaria* spp., *Macrocystis pyrifera*), as well as sessile animals (e.g. mussel, barnacle, polychaete and porifera) that live in aquatic environments, do not suffer autotoxicity at all because their wastes are continuously removed by flushing water (Bonanomi et al., 2010). In fact, stable and self-perpetuating monospecific stand in nature occurs only in aquatic environment where the plant mineral nutrition is almost completely decoupled from decaying of conspecific debris, thus nullifying their autotoxic impact. In contrast, in terrestrial ecosystems, autotoxicity accumulates in the close proximity of the producing

individual, resulting in a patchy, localized negative feedback neighborhood. Finally, we believe that SS results from the unavoidable constraint of localized waste accumulation combined with the sessile nature of terrestrial plants.

4.4. Synergic interaction between pathogens and toxins

SS is a complex, multifaceted phenomenon determined by an overall deterioration of soil quality. Unfortunately, most of available studies focused on a single causal factor to explain SS, with plant pathologists addressing the role of soilborne pathogens and parasitic nematodes, organic chemists searching for toxic molecules, and agronomists looking for depletion and imbalance of nutritional factors (Table 1). Only few researchers explored the possibility that multiple stress factors, both biotic and abiotic, may contribute to the development of SS.

More than 50 years ago, Patrick and co-workers (1963) proposed that toxic compounds, either released by roots during exudation or during decomposition of plant residues, promote the activity of soilborne pathogens by weakening the plant, thus reducing their fitness and resistance to pathogens. Since then, several studies confirmed the Patrick's hypothesis (Table 2). Xia et al. (2015) found that regeneration failure of Chinese fir (*Cunninghamia lanceolata*) in monospecific forest plantations was due to the strong concentration of cyclic dipeptides produced and released by the plant itself. These compounds not only were autotoxic for seedling roots, but also altered the microbial community composition, favouring the build-up of soilborne pathogens. A study carried out in hydroponic conditions, reported that the cinnamic acid contained in the root exudates of cucumber (*Cucumis sativus*) predisposed the roots to infection by the pathogen *Fusarium oxysporum* f. sp. *cucumerinum* through a direct biochemical and physiological effect (Ye et al., 2004). Studying the *Asparagus officinalis* replant disease problem, Hartung and Stephens (1983) reported that soil amendment with dried crown and root tissues promoted seedling attack by *F. oxysporum* f. sp. *asparagi* and *F. moniliforme*. Similarly, Bonanomi et al. (2007) found that tomato (*Solanum lycopersicon*) wilting caused by *F. oxysporum* f. sp. *lycopersici* increased when the soil was amended with tomato leaves that, *in vitro*, showed an autotoxic effect. Few years later, these finding were extended to polyphagous soilborne pathogens. Specifically, Bonanomi et al. (2011a) reported that alfalfa (*Medicago sativa*) seedling damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* dramatically increased when soil was amended with alfalfa residues, an organic material having a strong autotoxic effect. The authors suggest that *P. ultimum* and *R. solani*, having the ability to growth saprophytically on crop residues, increased their potential inoculum, and this impacted the disease incidence and severity. Benizri et al. (2005) studied the bacterial community structure in both healthy and sick soils

aiming to understand the role of microbes and toxins in the peach replant disease. The authors observed a higher abundance of *Bacillus* strains in sick rather than in healthy soil, whereas an opposite trend was found for *Pseudomonas* strains. In addition, more than 60% of the strains isolated from the sick soil were able to produce cyanides as secondary metabolite. These results suggested that peach replant disease can be caused directly by the presence of pathogenic microorganisms, and indirectly by the release of toxic compounds during the decomposition of peach root residues (Benizri et al., 2005; Yang et al., 2012).

Recently, Mazzoleni et al. (2015a) hypothesized that weakening of plants as a result of exposure to extracellular self-DNA with autotoxic effects, could increase its susceptibility to subsequent pathogen attack. However, the role of self-DNA in affecting plant-pathogen interactions is far to be fully understood, also in the light of the findings of Wen et al. (2009). Using the *Pisum sativum-Nectria haematococca* pathosystem, these authors demonstrated that exDNA is a component of root cap slime and is necessary for the protection of root tip from pathogen attack. The selective elimination of exDNA from the rhizosphere by the application of DNase I, resulted in the loss of root tip resistance, driving to fungal infection. These apparently contradictory findings underline the importance of further studies to clarify the role of exDNA on plant-pathogen interactions.

Finally, it should be pointed out that none of the studies listed in Table 2 demonstrated an effective reduced plant resistance after the application of purified chemical compounds or plant residues with autotoxic effect. Future studies based on physiological, proteomic and transcriptomics approaches are needed to test this hypothesis.

5. Overcoming soil sickness and negative plant-soil feedback

Farmers face SS since the time of ancient Greek and Roman Empire, being so forced to develop agronomic practices to overcome this problem. On the other hand, terrestrial plants that live in natural ecosystems evolved under the selective pressure of NPSF, driving single individuals to move in time and space to avoid their own "home" soil. The comparison between management strategies aimed at overcoming SS in agro-ecosystems and plant behavior to avoid NPSF in natural plant communities provide interesting parallelisms (Table 3).

5.1. Crop rotation

Crop rotation is probably the most ancient agronomic method to overcome SS, being already cited by Columella (4 – 70 AD) more than 2,000 years ago. Crop rotation alleviates SS as it decreases the pathogen inoculum, and it reduces the effect of autotoxic compounds in the soil (Curl, 1963;

Huang et al., 2013; Zucconi, 2003). These effects are achieved by mixing the residues of different crops that succeed in time. Plant debris of some *Brassica* species are also highly effective in controlling some soilborne pathogens and parasitic nematodes thanks to their high content in glucosinolates (Lawrence and Matthiessen, 2004; Lazzeri et al., 2004). Rotation between cereal crops, such as wheat and maize, with nitrogen-fixing legumes (e.g. clover, alfalfa, soybean, etc.) is probably one of the most common practices worldwide to alleviate SS. Alternation of grasses and nitrogen-fixing legumes in natural grassland has been also reported (Turkington et al., 1977). Although the effectiveness of this rotation is commonly linked to an increased content of nitrogen in the soil, changes in soil microbiota and the alleviation of autotoxicity could be important as well.

In natural plant communities, alternation of species in space and time is the most common strategy to escape NPSF (Table 3). In fact, alternation of different species has been observed in forest (Fox, 1977), shrubland (Watt 1947) and grassland (Maarel and Sykes, 1993). In general, plants move away from sick, "home soil" previously occupied by conspecific by mean of seed dispersal or clonal propagation. The resulting spatial and temporal patterns depend on the growth form, resulting in the Janzen-Connell distribution for trees and shrubs, "ring" and "wave" growth in perennial clonal plants, and a rapid turnover in annual species (Vincenot et al., 2017). In all cases, however, the plant community behaves in response to NPSF producing a sort of natural, self-emerging alternation or rotation of different species in time and space. We are still far from a comprehensive understanding of dynamics and mechanisms behind natural alternations, but a better comprehension of these processes would be invaluable for modern and sustainable agriculture.

5.2. Polyculture and organic amendment

Polyculture, the contemporaneous cultivation of different plant species in the same field, is the most effective system to avoid SS. In fact, polyculture does not allow the development of SS because this require a certain time of monoculture to build-up in soil. Polyculture substantially mimics a natural ecosystem where different plant species coexist in mixed communities. Theoretical as well empirical studies demonstrated that polyculture reduces the incidence of diseases and pests by means of "herd" protection (Matson et al., 1997; Wills et al., 1997; Boudreau, 2013). In this model, heterospecific crowding protects different species because each individual is hidden by the surrounding vegetation, resulting in fewer host-pathogen compatible interactions. Polyculture can avoid the build-up of SS also trough a "dilution effect" of autotoxic compounds. In monospecific stand, single species plant debris accumulate punctually, and autotoxicity progressively increases. Conversely, in a multiple species stand different plant residues are mixed, resulting in the dilution of

autotoxic compounds. Thus, despite some unavoidable effects at the individual level, there is a positive outcome at the community level. Interestingly, under these assumptions, competitive effects occur on a short-term scale, but positive reciprocal species interactions emerge only if all species suffer from negative feedback (Bonanomi et al., 2005c). The role of mixing plant debris has been extensively studied in relation to decaying rate and nutrient dynamics (review in Gartner and Cardon, 2004; Hättenschwiler et al., 2005), while no data are available about its feedback effect on plant growth.

Unfortunately, polyculture cannot be applied in most of intensive agricultural systems because of their complex management and the large amount of manpower required. A practical method to alleviate SS is the application of exogenous organic amendments. By this technique, a certain amount of organic matter is applied to soil to improve physical, chemical and biological properties (Bulluck et al., 2002; Diacono and Montemurro, 2010; Reeves, 1997; Stark et al., 2007). Organic amendment operates by "diluting" the autotoxic effect of crop residues that are mixed with organic matter of different nature. However, the effectiveness of organic amendment to overcome SS depends on the amount of organic carbon applied and on the chemical quality of the amendment itself (Zucconi, 2003).

5.3. Replacing sick soil

Removal of sick soil and its replacement with "fresh" one is a simple and effective method to overcome SS (Zucconi, 2003). For obvious economic reasons this method cannot be applied in field conditions, being limited to some cultivations in nurseries and in public as well private gardens. Interestingly, the replacement of sick soil has been observed under specific ecological condition in natural ecosystems. Studying sand dune communities in North Europe, Van der Putten et al. (1993) reported that the accumulation of soilborne pathogens and plant-parasitic nematodes are responsible for NPSF in Marram grass. However, detrimental soil conditions to this plant do not develop until sand accretion from the near seashore occurs. In fact, every year the shoot of this plant is buried by 20-100 cm of sand blow material. Marram grass is adapted to burial, a stress that will kill most of other plants, thanks to creeping rhizomes. Moreover, the rhizome benefits of the "fresh" sand, free from any pathogen, thus remaining vigorous. A similar behaviour has been described for several grasses that live in sandy deserts (Danin et al., 2012).

Soil accretion commonly occurs during flooding along river banks. Bonanomi et al. (2014), studying the perennial sedge *Scirpus holoshoenus* that live in Italian river banks, found that this plant generates two types of tussocks according to different environmental conditions: i. loose tussocks

with low tiller density and central "dieback"; ii. compact tussocks with high tiller density and concave surface, without central "dieback". After extensive plant excavation and analysis of rhizome architecture, the authors showed that in the first case plants form "rings" because the rhizomes grow radially and did not resprout in the inner, "dieback" area of the clone. Such rhizome architecture was found only in not inundated grasslands. In the case of compact and concave tussocks, the rhizome is able to grow vertically, following substrate accretion that occurred during previous flooding events. Orthotropic rhizome grown following tussock burial demonstrates that the new accreted soil, free from pathogens and toxins, is suitable for root development, differently from the soil present in the inner area of the clone.

5.4. Removal of soil toxins

Selective removal of phytotoxic compounds has been proposed as another strategy to alleviate SS and NPSF. In this regard, activated carbon (AC) has been used because of its strong capacity to adsorb organic chemicals, including pollutants and allelopathic compounds (Downie et al., 2009; Hille and den Ouden, 2005). AC sorption capability has been exploited in soilless system as well as in field conditions. For example, application of AC to circulating solution in hydroponic systems increases the productivity in tomato, and in asparagus of 15–30% (Asao et al., 2003; Yu et al., 1993; Yu and Matsui, 1994). Noteworthy, nutrient solutions collected after one cultivation cycle, resulted toxic for the seedling of conspecific plants, but no toxic effect was observed when the solution was treated with AC. Similar findings were reported for several cucurbit crops in soilless systems (Yu, 2001). The use of AC in sick soil was less effective, with a significant alleviation of the replant problem in *Asparagus* (Motoki et al., 2006), but having negligible effects on other species (Petermann et al., 2008). The complex interaction between toxins, native organic matter, soil microbiota and AC may explain the variable results achieved in field conditions compared to soilless systems. In natural soils, biochar can accumulate as a result of natural or anthropogenic burning of vegetation (DeLuca et al., 2006; Glaser and Birk, 2012). Burning of crop residues or natural vegetation produces highly heterogeneous materials, ranging from little affected plant tissues, to a variety of charred substrates, up to mineral ash (González-Pérez et al., 2004). The amount and chemical properties of burnt organic residues depends on both the biochemical composition of the plant tissues (Knicker, 2007), and the fire intensity that is controlled by pre-fire biomass moisture, fuel spatial arrangement and local microclimatic conditions (Certini, 2005). For instance, in the Amazonian basin pre-Columbian populations developed the so-called "*terra preta*" or "*dark earth*" by repeating cycles of fire and cultivation, i.e. the slash-and-char cultivation system (Glaser and Birk, 2012). The described "*terra*

preta" contains very large amounts of biochar, reaching also 10-40% of the whole soil (Kammann et al., 2016). The accumulation of biochar and other pyrogenic organic materials transformed a poorly nutritious and highly weathered acidic soil into a fertile one, capable of sustaining mono-cropping of maize and other crops for centuries (Kammann et al., 2016).

Soil flooding has been applied by farmers to overcome SS in Japan and China. Periodical soil flooding, for several weeks or some months can potentially leachate water soluble autotoxic substances and control some soilborne pathogens (Newhall, 1955; Nie et al., 2009). For instance, rice (*Oryza sativa*) was subjected to a more intense yield decline in monoculture under aerobic cultivation, compared to flooded conditions (Nie et al., 2007; Peng et al., 2006). Moreover, periodical flooding of aerobic rice cultivation alleviated the symptoms of SS (Nie et al., 2009). Flooding has been proved to be effective against SS in field conditions also for sugarcane (Chou, 1995), but no studies investigated the real movement, and the eventual leaching, of the putative autotoxic compounds in the field. The effectiveness of flooding in overcoming, or in alleviating SS, is consistent with the lack of NPSF in plants that live in aquatic ecosystems (Bonanomi et al., 2010). As already stated, stable and self-perpetuating monospecific stand in nature can be observed only in aquatic environments, with examples that include floating (e.g. *Eichhornia crassipes*, *Lemna* spp., *Pistia* spp.), as well herbaceous perennial (*Phragmites autsralis*, *Posidonia* spp.) and woody (*Avicennia* spp., *Rhizophora* spp.) plants rooted in the sediments. In aquatic ecosystems, plants do not suffer autotoxicity and, therefore, NPSF because their wastes (i.e. leaf and root residues) are continuously removed by flushing water. Moreover, in such aquatic ecosystems, plant mineral nutrition is almost completely decoupled from decaying of conspecific debris because roots absorb nutrients from the water, thus the potential autotoxic impact of organic residues are nullified. Noteworthy, periodical "die-back" due to litter autotoxicity has been observed also in aquatic systems, but only after reductions of water regimes that, presumably, do not allow an efficient removal of decaying debris and their autotoxic by-products. Examples include the "die-back" of *Phragmites autsralis* (Armstrong and Armstrong, 2001; Van Der Putten, 1997), *Typha latifolia* (McNaughton, 1968), as well as several seagrasses and seaweed (e.g. Borum et al., 2005; Frederiksen et al., 2007). In other words, we speculate that human-managed monocultures can only be sustained in the long-term by decoupling the resource acquirement from autotoxic plant debris. This can be achieved by either removal of crop residues (e.g. by using burning, selective removal, etc.), or mixing the residues through crop rotation or consociation, or leaching autotoxic factors trough flooding or biochar sequestration.

6. Conclusions and future perspectives

Soil sickness affects most of the major field crops, ornamental and horticultural species, as well as orchards. Intensive cultivations systems, based on monoculture, undeniably lead to the development of detrimental soil conditions that limit the cultivation of the same crop. There is an urgent need to find sustainable strategies to avoid or at the least alleviate SS, also considering the progressive ban of fumigants, that actually are the most effective method to temporary overcome the problem, and allow the cultivations. In this regard, a better understanding of the causes of SS is a necessary step to develop eco-friendly solutions. Evidence from both agro-ecosystems and natural plant communities undoubtedly ruled-out the nutrient deficiency as a primary causal factor of SS. The massive use of mineral fertilizers, especially in intensive cultivation systems, appears an incorrect strategy because it does not aim to solve the cause of SS, but it actually exacerbates the decline in soil quality often by inducing acidification and salinization (Bonanomi et al., 2011b; Ju et al., 2007).

Soilborne pathogens have been often isolated from symptomatic plants and many autotoxic compounds have been identified and quantified from sick soil. However, both the pathogenic and autotoxicity hypotheses are still unable to fully explain the species-specificity, and the long durability of soil sickness in field conditions. In other words, the relative role of detrimental microbial consortia, and autotoxic factors in SS is far to be completely understood. Determining which microbes determine the observed plant decline require testing Koch's postulates, which are based on the selective exclusion of all possible microbes one by one and adding them back again one by one. The enormous diversity of soil microbiota makes this approach practically infeasible, especially if all possible interactions are considered. Innovative approaches are required to circumvent this methodological limitation. Recently, Van der Putten (2017) described soil microbiome as an orchestra where different microbial groups contribute to the whole symphony. Then, which microbial group creates the dissonance in sick soil? Studies from natural ecosystems suggest that an unbalance between mycorrhizal fungi and soilborne pathogens is a pivotal factor. More holistic approaches, for instance based on metagenomics, would be very useful in agro-ecosystems where the reductionist approach does not seem to give clear cut answers. The recent discovery that exDNA has self-inhibitory effects renewed the interest on the autotoxicity hypothesis, nevertheless this is a totally new topic, and more solid and systematic field investigations are needed.

7. References

Abiven, S., Menasseri, S., Chenu, C., 2009. The effects of organic inputs over time on soil aggregate stability–A literature analysis. *Soil Biology and Biochemistry* **41**: 1–12.

Adachi, N., Terashima, I., Takahashi, M., 1996. Mechanisms of central die-back of *Reynoutria japonica* in the volcanic desert on Mt. Fuji. A stochastic model analysis of rhizome growth. *Annals of botany* **78**: 169–179.

Alström, S., 1992. Saprophytic soil microflora in relation to yield reductions in soil repeatedly cropped with barley (*Hordeum vulgare* L.). *Biology and Fertility of Soils* **14**: 145–150.

An, M., Pratley, J.E., Haig, T., 2001. Phytotoxicity of *Vulpia Residues*. IV. Dynamics of allelochemicals during decomposition of *Vulpia residues* and their corresponding phytotoxicity. *Journal of Chemical Ecology* **27**: 395–407.

Armstrong, J., Armstrong, W., 2001. An overview of the effects of phytotoxins on *Pragmites australis* in relation to die-back, in: *Aquatic Botany* **69**: 251–268.

Artursson, V., Finlay, R.D., Jansson, J.K., 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. *Environmental microbiology* **8**: 1–10.

Asaduzzaman, M., Asao, T., 2012. Autotoxicity in beans and their allelochemicals. *Scientia Horticulturae* **134**: 26–31.

Asaduzzaman, M., Kobayashi, Y., Isogami, K., Tokura, M., Tokumasa, K., Asao, T., 2012. Growth and yield recovery in strawberry plants under autotoxicity through electrodegradation. *European Journal of Horticultural Science* **77**: 58–67.

Asao, T., Hasegawa, K., Sueda, Y., Tomita, K., Taniguchi, K., Hosoki, T., Pramanik, M.H.R., Matsui, Y., 2003. Autotoxicity of root exudates from taro. *Scientia Horticulturae* **97**: 389–396.

Asao, T., Kitazawa, H., Ban, T., Pramanik, M.H.R., Matsui, Y., Hosoki, T., 2004. Search of autotoxic substances in some leaf vegetables. *Journal Of The Japanese Society For Horticultural Science* **73**: 247–249.

Asao, T., Kitazawa, H., Ushio, K., Sueda, Y., Ban, T., Pramanik, M.H.R., 2007. Autotoxicity in Some Ornamentals with the Means to Overcome It. *Horticultural Science* **42**: 1346–1350.

Augspurger, C.K., 1984. Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps, and pathogens. *Ecology* **65**: 1705–1712.

Barbero, F., Guglielmotto, M., Capuzzo, A., Maffei, M.E. (2016). Extracellular Self-DNA (esDNA), but not heterologous plant or insect DNA (etDNA), induces plasma membrane depolarization and calcium signaling in lima bean (*Phaseolus lunatus*) and maize (*Zea mays*). *International Journal of Molecular Sciences* **17**: 1659.

Bartelt-Ryser, J., Joshi, J., Schmid, B., Brandl, H., Balser, T., 2005. Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. *Perspectives in Plant Ecology Evolution and Systematics* **7**: 27–49.

Bastida, F., Zsolnay, A., Hernández, T., García, C., 2008. Past, present and future of soil quality indices: A biological perspective. *Geoderma* **147**: 159–171.

Bekunda, M., 1999. Farmers' responses to soil fertility decline in banana-based cropping systems of Uganda. *Managing Africa's Soils*. IIED-Drylands Programme.

Bellingham, P.J., Walker, L.R., Wardle, D.A., 2001. Differential facilitation by a nitrogen-fixing shrub during primary succession influences relative performance of canopy tree species. *Journal of Ecology* **89**: 861–875.

Benedict, H.M., 1941. The inhibiting effect of dead roots on the growth of bromegrass. *Agronomy Journal* **33**: 1108–1109.

Ben-Hammouda, M., Ghorbal, H., Kremer, R.J., Oueslatt, O., 2002. Autotoxicity of Barley. *Journal of Plant Nutrition* **25**: 1155–1161.

Benizri, E., Piutti, S., Verger, S., Pagès, L., Vercambre, G., Poessel, J.L., Michelot, P., 2005. Replant diseases: Bacterial community structure and diversity in peach rhizosphere as determined by metabolic and genetic fingerprinting. *Soil Biology and Biochemistry* **37**: 1738–1746.

Bennett, A.J., Bending, G.D., Chandler, D., Hilton, S., Mills, P., 2012. Meeting the demand for crop production: The challenge of yield decline in crops grown in short rotations. *Biological Reviews* **87**: 52–71.

Bennett, J.A., Maherali, H., Reinhart, K.O., Lekberg, Y., Hart, M.M., Klironomos, J., 2017. Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* **355**: 181–184.

Bever, J.D., 1994. Feedback between plants and their soil communities in an old field community. *Ecology* **75**: 1965–1977.

Bever, J.D., Westover, K.M., Antonovics, J., 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *The Journal of Ecology* **85**: 561.

Bever, J.D., Broadhurst, L.M., Thrall, P.H. 2013. Microbial phylotype composition and diversity predicts plant productivity and plant–soil feedbacks. *Ecology Letters* **16**: 167-174.

Bezemer, T.M., Lawson, C.S., Hedlund, K., Edwards, A.R., Brook, A.J., Igual, J.M., Mortimer, S.R., Van Der Putten, W.H., 2006. Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. *Journal of Ecology* **94**: 893–904.

Blok, W.J., Bollen, G.J., 1996. Interactions of asparagus root tissue with soil microorganisms as a factor in early decline of asparagus. *Plant pathology* **45**: 809–822.

Blum, U., Shafer, S.R., Lehman, M.E., 1999. Evidence for inhibitory allelopathic interactions involving phenolic acids in field soils: Concepts vs. an experimental model. *Critical Reviews in Plant Sciences* **18**: 673–693.

Blundell, A.G., Peart, D.R., 2004. Seedling recruitment failure following dipterocarp mast fruiting. *Journal of Tropical Ecology* **20**: 229–231.

Bodker, L., Leroul, N., 1993. Influence of pea cropping history on disease severity and yield depression. *Plant Disease* **77**: 896–900.

Bonanomi, G., Allegrezza, M., 2004. Effetti della colonizzazione di *Brachypodium rupestre* (Host) Roemer et Schultes sulla diversità di alcune fitocenosi erbacee dell'Appennino centrale. *Fitosociologia* **41**: 51–69.

Bonanomi, G., Legg, C., Mazzoleni, S. 2005a. Autoinhibition of germination and seedling establishment by leachate of *Calluna vulgaris* leaves and litter. *Community Ecology* **6**: 203-208.

Bonanomi, G., Rietkerk, M., Dekker, S.C., Mazzoleni, S. 2005b. Negative plant–soil feedback and positive species interaction in a herbaceous plant community. *Plant Ecology* **181**: 269-278.

Bonanomi, G., Giannino, F., Mazzoleni, S. 2005c. Negative plant–soil feedback and species coexistence. *Oikos* **111**: 311-321.

Bonanomi, G., Sicurezza, M.G., Caporaso, S., Esposito, A., Mazzoleni, S., 2006. Phytotoxicity dynamics of decaying plant materials. *New Phytologist* **169**: 571–578.

Bonanomi, G., Del Sorbo, G., Mazzoleni, S., Scala, F., 2007. Autotoxicity of decaying tomato residues affects susceptibility of tomato to fusarium wilt. *Journal of Plant Pathology* **89**: 219–226.

Bonanomi, G., Rietkerk, M., Dekker, S.C., Mazzoleni, S., 2008. Islands of fertility induce co-occurring negative and positive plant-soil feedbacks promoting coexistence. *Plant Ecology* **197**: 207–218.

Bonanomi, G., Incerti, G., Capodilupo, M., Mazzoleni, S. 2010. Rare self-facilitation in terrestrial plants as compared to aquatic sessile organisms: empirical evidences and causal mechanisms. *Community Ecology* **11**: 148-159.

Bonanomi, G., Antignani, V., Barile, E., Lanzotti, V., Scala, F., 2011a. Decomposition of *Medicago sativa* residues affects phytotoxicity, fungal growth and soil-borne pathogen diseases. *Journal of Plant Pathology* **93**: 57–69.

Bonanomi, G., D'Ascoli, R., Antignani, V., Capodilupo, M., Cozzolino, L., Marzaioli, R., Puopolo, G., Rutigliano, F.A., Scelza, R., Scotti, R., Rao, M.A., Zoina, A., 2011b. Assessing soil quality under intensive cultivation and tree orchards in Southern Italy. *Applied Soil Ecology* **47**: 184–194.

Bonanomi, G., Incerti, G., Barile, E., Capodilupo, M., Antignani, V., Mingo, A., Mazzoleni, S. 2011c. Phytotoxicity, not nitrogen immobilization, explains plant litter inhibitory effects: evidence from solid-state ^{13}C NMR spectroscopy. *New Phytologist* **191**: 1018-1030.

Bonanomi, G., Incerti, G., Stinca, A., Cartenì, F., Giannino, F., Mazzoleni, S. 2014. Ring formation in clonal plants. *Community Ecology* **15**: 77-86.

Bonanomi, G., De Filippis, F., Cesarano, G., La Storia, A., Ercolini, D., Scala, F. 2016. Organic farming induces changes in soil microbiota that affect agro-ecosystem functions. *Soil Biology and Biochemistry* **103**: 327-336.

Bonner, J., Galston, A.W., 1944. Toxic substances from the culture media of guayule which may inhibit growth. *Botanical Gazette* **106**: 185–198.

Borner, H., 1959. The apple replant problem. I. The excretion of phlorizin from apple root residues. *Contrib. Boyce Thompson Inst* **20**: 39–56.

Börner, H., 1960. Liberation of organic substances from higher plants and their role in the soil sickness problem. *The Botanical Review* **26**: 393–424.

Borum, J., Pedersen, O., Greve, T.M., Frankovich, T.A., Zieman, J.C., Fourqurean, J.W., and Madden, C.J. 2005. The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass, *Thalassia testudinum*. *Journal of Ecology* **93**: 148-158.

Boudreau, M.A. 2013. Diseases in intercropping systems. *Annual Review of Phytopathology* **51**: 499-519.

Bronick, C., Lal, R., 2005. Soil structure and management: a review. *Geoderma* **124**: 3–22.

Bu, Y.Q., Xue, Y.R., Gan, L.S., Liu, C.H., Shan, Z.J., 2014. *Enterobacter amnigenus W5*: A potential causal agent for *Rehmannia glutinosa* replant problem. *Allelopathy Journal* **34**: 4693.

Bulluck, L.R., Brosius, M., Evanylo, G.K., Ristaino, J.B., 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. *Applied Soil Ecology* **19**: 147–160.

Burger, W.P., Small, J.G.C., 1983. Allelopathy in citrus orchards. *Scientia Horticulturae* **20**: 361–375.

Canals, R.M., Emeterio, L.S., Peralta, J., 2005. Autotoxicity in *Lolium rigidum*: Analyzing the role of chemically mediated interactions in annual plant populations. *Journal of Theoretical Biology* **235**: 402–407.

Castellanos, E.M., Figueroa, M.E., Davy, A.J., 1994. Nucleation and facilitation in saltmarsh succession: interactions between *Spartina maritima* and *Arthrocnemum perenne*. *Journal of Ecology* 239–248.

Certini, G., 2005. Effects of fire on properties of forest soils: a review. *Oecologia* **143**: 1–10.

Chang, C.-F., Suzuki, A., Kumai, S., Tamura, S., 1969. Chemical Studies on "Clover Sickness." *Agricultural and Biological Chemistry* **33**: 398–408.

Chapin, F.S., Walker, L.R., Fastie, C.L., Sharman, L.C., 1994. Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. *Ecological Monographs* **64**: 149–175.

Chaturvedi, S.N., Muralia, R.N., 1975. Germination inhibitors in some umbellifer seeds. *Annals of botany* **39**: 1125–1129.

Chen, L., Wang, S., Yu, X., 2005. Effects of Phenolics on seedling growth and 15N nitrate absorption of *Cunninghamia lanceolata*. *Allelopathy Journal* **15**: 57–65.

Chen, T., Lin, S., Wu, L. K., Lin, W.X., Sampietro, D.A. 2015. Soil sickness: current status and future perspectives. *Allelopathy Journal* **36**: 167-196.

Chon, S.U., Choi, S.K., Jung, S., Jang, H.G., Pyo, B.S., Kim, S.M., 2002. Effects of alfalfa leaf extracts and phenolic allelochemicals on early seedling growth and root morphology of alfalfa and barnyard grass. *Crop Protection* **21**: 1077–1082.

Chou, C., Lin, H., 1976. Autointoxication mechanism of *Oryza sativa*. *J.Chem.Ecol.* **2**: 353–367.

Chou, C.-H., Waller, G.R., 1980. Possible allelopathic constituents of *Coffea arabica*. *Journal of chemical ecology* **6**: 643–654.

Chou, C.H. 1995. *Allelopathy and sustainable agriculture*. ACS Symposium Series, Vol. 582, pp. 211-223.

Collina, F., Zucconi, F., 1967. Contributo allo studio dell'azione di residui di colture diverse sullo sviluppo del pesco. *Rivista di ortoflorofrutticoltura italiana* 386–406.

Crump, D.H., Kerry, B.R., 1987. Studies on the population dynamics and fungal parasitism of *Heterodera schachtii* in soil from a sugar-beet monoculture. *Crop Protection* **6**: 49–55.

Curl, E.A., 1963. Control of plant diseases by crop rotation. *The Botanical Review* **29**: 413–479.

Curtis, J.T., Cottam, G., 1950. Antibiotic and autotoxic effects in prairie sunflower. *Bulletin of the Torrey botanical club* **77**: 187–191.

Danin, A. 2012. *Plants of desert dunes*. Springer Science and Business Media.

Delogu, G., Faccini, N., Alberici, R., Gianinetti, A., Stanca, A.M., 2003. Soil-borne viruses of barley seriously affect plant growth and grain yield in a monocropping system. *Cereal Research Communications* **31**: 137–144.

DeLuca, T.H., MacKenzie, M.D., Gundale, M.J., Holben, W.E., 2006. Wildfire-produced charcoal directly influences nitrogen cycling in ponderosa pine forests. *Soil Science Society of America Journal* **70**: 448–453.

Diacono, M., Montemurro, F., 2010. Long-term effects of organic amendments on soil fertility. A review. *Agronomy for Sustainable Development* **30**: 401–422.

Doran, J.W., Parkin, T.B., 1994. Defining and assessing soil quality. *Defining soil quality for a sustainable environment* 1-21.

Downie A., Crosky A., Munroe P., 2009. Physical properties of biochar. In: Lehmann J., Joseph S. (eds). *Biochar for Environmental Management: Science and Technology*. Earthscan, London, UK.

Drewry, J.J., Cameron, K.C., Buchan, G.D., 2008. Pasture yield and soil physical property responses to soil compaction from treading and grazing-a review. *Australian Journal of Soil Research* **46**: 237.

Ehrenfeld, J. G., Ravit, B., and Elgersma, K., 2005. Feedback in the plant-soil system. *Annual Review of Environment and Resources* **30**: 75-115.

Elmer, W.H., Pignatello, J.J., 2011. Effect of biochar amendments on mycorrhizal associations and *Fusarium* crown and root rot of asparagus in replant soils. *Plant Disease* **95**: 960–966.

Endeshaw, S.T., Lodolini, E.M., Neri, D., 2015. Effects of olive shoot residues on shoot and root growth of potted olive plantlets. *Scientia Horticulturae* **182**: 31–40.

Facelli, J.M., Brock, D.J., 2000. Patch dynamics in arid lands: localized effects of *Acacia papyrocarpa* on soils and vegetation of open woodlands of South Australia. *Ecography* **23**: 479–491.

Fatemi, J., 1980. The role of Phytophthora species in almond decline in Iran. *Journal of Phytopathology* **99**: 97–100.

Fitter, A., 2003. Making allelopathy respectable. *Science* **301**: 1337–1338.

Florence, R.G., 1965. Decline of old-growth redwood forests in relation to some soil microbiological processes. *Ecology* **46**: 52–64.

Florence, R.G., Crocker, R.L., 1962. Analysis of blackbutt (*Eucalyptus pilularis* Sm.) seedling growth in a blackbutt forest soil. *Ecology* **43**: 670–679.

Fox, J.F., 1977. Alternation and coexistence of tree species. *The American Naturalist* **111**: 69–89.

Frederiksen, M.S., Holmer, M., Díaz-Almela, E., Marba, N., Duarte, C.M. 2007. Sulfide invasion in the seagrass *Posidonia oceanica* at Mediterranean fish farms: assessment using stable sulfur isotopes. *Marine Ecology Progress Series* **345**: 93-104.

Gartner, T.B., Cardon, Z.G., 2004. Decomposition dynamics in mixed-species leaf litter. *Oikos* **104**: 230–246.

Gentry, L.F., Ruffo, M.L., Below, F.E., 2013. Identifying factors controlling the continuous corn yield penalty. *Agronomy Journal* **105**: 295–303.

Glaser, B., Birk, J.J., 2012. State of the scientific knowledge on properties and genesis of Anthropogenic Dark Earths in Central Amazonia (terra preta de Índio). *Geochimica et Cosmochimica acta* **82**: 39–51.

González-Pérez, J.A., González-Vila, F.J., Almendros, G., Knicker, H. 2004. The effect of fire on soil organic matter: a review. *Environment International* **30**: 855-870.

Grente, J., 1963. Walnut decline.

Gresta, F., Santonoceto, C., Avola, G., 2016. Crop rotation as an effective strategy for saffron (*Crocus sativus* L.) cultivation. *Scientia Horticulturae* **211**: 34–39.

Gupta, A.K., 2004. Origin of agriculture and domestication of plants and animals linked to early Holocene climate amelioration. *Current Science*.

Han, L., Wang, S., Ju, H., Yan, F., Li, G., Liu, J., Yan, J., 2002. Identification and study on allelopathy of soybean root exudates. *Soybean science*.

Hao, Z.P., Wang, Q., Christie, P., Li, X.L., 2007. Allelopathic potential of watermelon tissues and root exudates. *Scientia Horticulturae* **112**: 315–320.

Harper, J.L., 1977. *Population Biology of Plants*. Vol. 892. London: Academic press.

Hartemink, A.E., Poloma, S., Maino, M., Powell, K.S., Egenae, J., O'Sullivan, J.N., 2000. Yield decline of sweet potato in the humid lowlands of Papua New Guinea. *Agriculture, Ecosystems and Environment* **79**: 259–269.

Hartung, A.C., Stephens, C.T., 1983. Effects of allelopathic substances produced by asparagus on incidence and severity of asparagus decline due to Fusarium crown rot. *Journal of Chemical Ecology* **9**: 1163–1174.

Hassan, N., Ah Elbehadl, I., Is Alsaadaw, I., 1989. Citrus Replant Problem in Iraq .3. Interactive Effect of Soil Fungi and Allelopathy. *Plant and Soil* **116**: 151–155.

Hättenschwiler, S., Tiunov, A. V., Scheu, S., 2005. Biodiversity and Litter Decomposition in Terrestrial Ecosystems. *Annual Review of Ecology, Evolution, and Systematics* **36**: 191–218.

Hayat, R., Ali, S., Amara, U., Khalid, R., Ahmed, I., 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology* **60**: 579–598.

He, C.N., Gao, W.W., Yang, J.X., Bi, W., Zhang, X.S., Zhao, Y.J., 2009. Identification of autotoxic compounds from fibrous roots of *Panax quinquefolium* L. *Plant and Soil* **318**: 63–72.

Hille M., Den Ouden J., 2005. Charcoal and activated carbon as adsorbate of phytotoxic compounds - a comparative study. *Oikos* **108**: 202-207.

Hilton, S., Bennett, A.J., Keane, G., Bending, G.D., Chandler, D., Stobart, R., Mills, P., 2013. Impact of shortened crop rotation of oilseed rape on soil and rhizosphere microbial diversity in relation to yield decline. *PLoS ONE* **8**: 1–12.

Himanen, M., Prochazka, P., Hänninen, K., Oikari, A. 2012. Phytotoxicity of low-weight carboxylic acids. *Chemosphere* **88**: 426-431.

Hodge, A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytologist* **162**: 9-24.

Hodge, A., Robinson, D., Fitter, A., 2000. Are microorganisms more effective than plants at competing for nitrogen? *Trends in Plant Science*.

Hoestra, H., 1965. *Thielaviopsis basicola*, a factor in the cherry replant problem in the Netherlands. *European Journal of Plant Pathology* **71**: 180-182.

Hoestra, H., 1968. Replant diseases of apple in the Netherlands. PhD Thesis. Veenman.

Hosomi, A., Uchiyama, T., 1998. Growth inhibiting factors in sick soil of fig orchards. *Journal of the Japanese Society for Horticultural Science* **67**: 44–50.

Howeler, R.H., Cadavid, L.F., 1990. Short-and long-term fertility trials in Colombia to determine the nutrient requirements of cassava. *Fertilizer Research* **26**: 61–80.

Howeler, R.H., 1991. Long-term effect of cassava cultivation on soil productivity. *Field Crops Research* **26**: 1–18.

Huang, X., Bie, Z., Huang, Y., 2010. Identification of autotoxins in rhizosphere soils under the continuous cropping of cowpea. *Allelopathy Journal* **25**: 383–392.

Huang, L.F., Song, L.X., Xia, X.J., Mao, W.H., Shi, K., Zhou, Y.H., Yu, J.Q., 2013. Plant-soil feedbacks and soil sickness: from mechanisms to application in agriculture. *Journal of Chemical Ecology* **39**: 232–242.

Huang, Y.Q., Han, X.R., Yang, J.F., Liang, C.H., Zhan, X.M., 2013. Autotoxicity of peanut and identification of phytotoxic substances in rhizosphere soil. *Allelopathy Journal* **31**: 297–308.

Ibekwe, A.M., Papiernik, S.K., Gan, J., Yates, S.R., Yang, C.H., Crowley, D.E., 2001. Impact of fumigants on soil microbial communities. *Applied and Environmental Microbiology* **67**: 3245–3257.

Incerti, G., Capodilupo, M., Senatore, M., Termolino, P., Scala, F., Mazzoleni, S., Bonanomi, G., 2013. Biochemical changes assessed by ^{13}C-CPMAS NMR spectroscopy control fungal growth on water extracts of decaying plant litter. *Mycoscience* **54**: 449–457.

Janvier, C., Villeneuve, F., Alabouvette, C., Edel-Hermann, V., Mateille, T., Steinberg, C., 2007. Soil health through soil disease suppression: which strategy from descriptors to indicators? *Soil Biology and Biochemistry* **39**: 1–23.

Janzen, D.H., 1970. Herbivores and the number of tree species in tropical forests. *The American Naturalist* **104**: 501–528.

Jennings, J.A., Nelson, C.J., 2002. Zone of autotoxic influence around established alfalfa plants. *Agronomy Journal* **94**: 1104–1111.

Jiang, G.-Y., Li, Y.-B., Liu, J.-G., 2013. Autotoxicity potential of cotton tissues and root exudates and identification of its autotoxins. *Allelopathy Journal* **32**: 279–288.

Johnston, A.E., 1986. Soil organic matter, effects on soils and crops. *Soil Use and Management* **2**: 97–105.

Jordan, N.R., Larson, D.L., Huerd, S.C., 2008. Soil modification by invasive plants: effects on native and invasive species of mixed-grass prairies. *Biological Invasions* **10**: 177–190.

Ju, X.T., Kou, C.L., Christie, P., Dou, Z.X., Zhang, F.S., 2007. Changes in the soil environment from excessive application of fertilizers and manures to two contrasting intensive cropping systems on the North China Plain. *Environmental Pollution* **145**: 497–506.

Kammann, C.I., Glaser, B., Schmidt, H.P. 2016. Combining biochar and organic amendments. Biochar in European Soils and Agriculture: Science and Practice **1**: 136-160.

Kardol, P., Martijn Bezemer, T., Van Der Putten, W.H., 2006. Temporal variation in plant-soil feedback controls succession. *Ecology Letters* **9**: 1080–1088.

Kardol, P., Cornips, N.J., Van Kempen, M.M.L., Bakx-Schotman, J.M.T., Van Der Putten, W.H., 2007. Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. *Ecological Monographs* **77**: 147–162.

Katznelson, J., 1972. Studies in Clover Soil Sickness. *Plant and Soil* **112**: 97–112.

Klironomos, J.N., 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* **417**: 67–70.

Knicker, H. 2007. How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. *Biogeochemistry* **85**: 91-118.

Kreye, C., Bouman, B.A.M., Faronilo, J.E., Llorca, L., 2009. Causes for soil sickness affecting early plant growth in aerobic rice. *Field Crops Research* **114**: 182–187.

Kukal, S.S., Singh, Y., Yadav, S., Humphreys, E., Kaur, A., Thaman, S., 2008. Why grain yield of transplanted rice on permanent raised beds declines with time? *Soil and Tillage Research* **99**: 261–267.

Kulmatiski, A., Beard, K.H., Stevens, J.R., Cobbold, S.M., 2008. Plant-soil feedbacks: A meta-analytical review. *Ecology Letters* **11**: 980–992.

Lambers, J.H.R., Clark, J.S., 2003. Effects of dispersal, shrubs, and density-dependent mortality on seed and seedling distributions in temperate forests. *Canadian Journal of Forest Research* **33**: 783–795.

Lanta, V., Dolezal, J., Samata, J., 2004. Vegetation patterns in a cut-away peatland in relation to abiotic and biotic factors: a case study from the Šumava Mts., Czech Republic. *Suo* **55**: 33–43.

Lanta, V., Janeček, Š., Doležal, J., 2008. Radial growth and ring formation process in clonal plant Eriophorum angustifolium on post-mined peatland in the Šumava Mts., Czech Republic. *Annales Botanici Fennici* **45**: 44–54.

Larkin, R.P. 2015. Soil health paradigms and implications for disease management. *Annual Review of Phytopathology* **53**: 199-221.

Larkin, R.P., Honeycutt, C.W., 2006. Effects of different 3-year cropping systems on soil microbial communities and rhizoctonia diseases of potato. *Phytopathology* **96**: 68–79.

Lawrence, L., Matthiessen, J., 2004. Biofumigation–Using brassica rotations to manage soil-borne pests and diseases. *Outlooks on Pest Management* **15**: 42–44.

Lazzeri, L., Curto, G., Leoni, O., Dallavalle, E., 2004. Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode *Meloidogyne incognita* (Kofoid et White) Chitw. *Journal of Agricultural and Food Chemistry* **52**: 6703–6707.

Levy-Booth, D.J., Campbell, R.G., Gulden, R.H., Hart, M.M., Powell, J.R., Klironomos, J.N., Pauls, K.P., Swanton, C.J., Trevors, J.T., Dunfield, K.E. 2007. Cycling of extracellular DNA in the soil environment. *Soil Biology and Biochemistry* **39**: 2977–2991.

Lewis, J.P., Stofella, S.L., Feldman, S.R., 2001. Monk s tonsure-like gaps in the tussock grass *Spartina argentinensis* (Gramineae). *Revista de biología tropical* **49**: 313–316.

Li, P., Wang, X., Li, Y., Wang, H., Liang, F., Dai, C., 2010a. The contents of phenolic acids in continuous cropping peanut and their allelopathy. *Acta Ecologica Sinica*.

Li, C., Li, X., Kong, W., Wu, Y., Wang, J., 2010b. Effect of monoculture soybean on soil microbial community in the Northeast China. *Plant and Soil* **330**: 423–433.

Li, Z.F., Yang, Y.Q., Xie, D.F., Zhu, L.F., Zhang, Z.G., Lin, W.X., 2012. Identification of autotoxic compounds in fibrous roots of Rehmannia (*Rehmannia glutinosa* Libosch.). *PLoS ONE* **7**: 1–8.

Li, X.G., Ding, C.F., Hua, K., Zhang, T.L., Zhang, Y.N., Zhao, L., Yang, Y.R., Liu, J.G., Wang, X.X., 2014a. Soil sickness of peanuts is attributable to modifications in soil microbes induced by peanut root exudates rather than to direct allelopathy. *Soil Biology and Biochemistry* **78**: 149–159.

Li, J.G., Ren, G. Di, Jia, Z.J., Dong, Y.H., 2014b. Composition and activity of rhizosphere microbial communities associated with healthy and diseased greenhouse tomatoes. *Plant and Soil* **380**: 337–347.

Li, X., Zhang, Y., Ding, C., Jia, Z., He, Z., Zhang, T., Wang, X., 2015. Declined soil suppressiveness to *Fusarium oxysporum* by rhizosphere microflora of cotton in soil sickness. *Biology and Fertility of Soils* **51**: 935–946.

Lopez-Iglesias, B., Olmo, M., Gallardo, A., Villar, R., 2014. Short-term effects of litter from 21 woody species on plant growth and root development. *Plant and Soil* **381**: 177–191.

Maarel, E., Sykes, M.T., 1993. Small-scale plant species turnover in a limestone grassland: the carousel model and some comments on the niche concept. *Journal of Vegetation Science* **4**: 179–188.

Mai, W.F., Abawi, G.S., 1978. Determining the cause and extent of apple, cherry, and pear replant diseases under controlled conditions. *Phytopathology* **68**: 1540–1544.

Mangan, S.A., Schnitzer, S.A., Herre, E.A., Mack, K.M.L., Valencia, M.C., Sanchez, E.I., Bever, J.D., 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* **466**: 752–5.

Manici, L.M., Ciavatta, C., Kelderer, M., Erschbaumer, G., 2003. Replant problems in South Tyrol: role of fungal pathogens and microbial population in conventional and organic apple orchards. *Plant and Soil* **256**: 315–324.

Manici, L.M., Caputo, F., Baruzzi, G., 2005. Additional experiences to elucidate the microbial component of soil suppressiveness towards strawberry black root rot complex. *Annals of applied biology* **146**: 421–431.

Manici, L.M., Kelderer, M., Franke-Whittle, I.H., Rühmer, T., Baab, G., Nicoletti, F., Caputo, F., Topp, A., Insam, H., Naef, A., 2013. Relationship between root-endophytic microbial communities and replant disease in specialized apple growing areas in Europe. *Applied Soil Ecology* **72**: 207–214.

Martin, V.L., McCoy, E.L., Dick, W. a., 1990. Allelopathy of Crop Residues Influences Corn Seed Germination and Early Growth. *Agronomy Journal* **82**: 555.

Martínez, M.A., Martínez, M.C., Bielza, P., Tello, J., Lacasa, A., 2011. Effect of biofumigation with manure amendments and repeated biosolarization on Fusarium densities in pepper crops. *Journal of Industrial Microbiology and Biotechnology* **38**: 3–11.

Matson, P.A., Parton, W.J., Power, A.G., Swift, M.J., 1997. Agricultural intensification and ecosystem properties. *Science* **277**: 504–509.

Mazzola, M., 1998. Elucidation of the microbial complex having a causal role in the development of apple replant disease in Washington. *Phytopathology* **88**: 930–8.

Mazzola, M., Manici, L.M., 2012. Apple replant disease: role of microbial ecology in cause and control. *Annual review of phytopathology* **50**: 45–65.

Mazzoleni, S., Bonanomi, G., Giannino, F., Rietkerk, M., Dekker, S.C., Zucconi, F., 2007. Is plant biodiversity driven by decomposition processes? An emerging new theory on plant diversity. *Community Ecology* **8**: 103–109.

Mazzoleni, S., Bonanomi, G., Incerti, G., Chiusano, M.L., Termolino, P., Mingo, A., Senatore, M., Giannino, F., Cartenì, F., Rietkerk, M., Lanzotti, V., 2015a. Inhibitory and toxic effects of extracellular self-DNA in litter: A mechanism for negative plant-soil feedbacks? *New Phytologist* **205**: 1195–1210.

Mazzoleni, S., Carteni, F., Bonanomi, G., Senatore, M., Termolino, P., Giannino, F., Incerti, G., Rietkerk, M., Lanzotti, V., Chiusano, M.L., 2015b. Inhibitory effects of extracellular self-DNA: A general biological process? *New Phytologist* **206**: 127–132.

McNaughton, S.J., 1968. Autotoxic Feedback in Relation to Germination and Seedling Growth in *Typha latifolia*. *Ecology* **49**: 367–369.

Meiners, S.J., 2014. Functional correlates of allelopathic potential in a successional plant community. *Plant Ecology* **215**: 661–672.

Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H., Raaijmakers, J.M. 2011 Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* **332**: 1097-1100.

Miller, D. A. (1996). Allelopathy in forage crop systems. *Agronomy Journal* **88**: 854-859.

Miller, H., Ikawa, M., Peirce, L., 1991. Caffeic acid identified as an inhibitory compound in asparagus root filtrate. *HortScience* **26**: 1525–1527.

Minuto, A., Minuto, G., Migheli, Q., Gullino, M.L., Garibaldi, A., 2002. The "stanchezza" (soil sickness) of sweet basil. *Phytopathologia Mediterranea* **41**: 85–91.

Mizutani, F., Itamura, H., Sugiura, A., Tomana, T., 1979. Studies on the soil sickness problem for peach trees, 2: Condensed tannins as growth inhibitors from peach roots. *Journal of the Japanese Society for Horticultural Science*.

Motoki, S., Nishihara, E., Kitazawa, H., Hiradate, S., Fujii, Y., Shinohara, Y., 2006. Activated carbon utilization to reduce allelopathy that obstructs the continuous cropping of asparagus (*Asparagus officinalis* L.). *Horticultural Research (Japan)*.

Naidu, R., Rengasamy, P., Sumner, M., 1995. Australian sodic soils: distribution, properties and management. East Melbourne, VIC: CSIRO Australia.

Nayyar, A., Hamel, C., Lafond, G., Gossen, B.D., Hanson, K., Germida, J., 2009. Soil microbial quality associated with yield reduction in continuous-pea. *Applied Soil Ecology* **43**: 115–121.

Neri, D., Bonanomi, G., Zucconi, F., Cozzolino, E., 1998. Studies on the organic matter supply to strawberry crop (Fragaria-Emilia Romagna). *Rivista di Frutticoltura e di Ortofloricoltura (Italy)*.

Newhall, A.G., 1955. Disinfestation of soil by heat, flooding and fumigation. *The Botanical Review* **21**: 189–250.

Nie, L., Peng, S., Bouman, B.A., Huang, J., Cui, K., Visperas, R.M., Park, H. K. 2007. Alleviating soil sickness caused by aerobic monocropping: responses of aerobic rice to soil oven-heating. *Plant and Soil* **300**: 185-195.

Nie, L., Xiang, J., Peng, S., Bouman, B.A.M., Huang, J., Cui, K., Visperas, R.M., 2009. Alleviating soil sickness caused by aerobic monocropping: Responses of aerobic rice to fallow, flooding and crop rotation. *Journal of Food, Agriculture and Environment* **7**: 723–727.

Nielsen, K.F., Cuddy, T.F., Woods, B.W., 1960. The influence of the extract of some crops and soil residues on germination and growth. *Canadian Journal of Plant Science* **40**: 188–197.

Nigh, E.L., 1990. Stress factors influencing Fusarium infection in asparagus. *Acta Horticulturae* 315–322.

Olff, H., Hoorens, B., De Goede, R.G.M., Van der Putten, W.H., Gleichman, J.M., 2000. Small-scale shifting mosaics of two dominant grassland species: the possible role of soil-borne pathogens. *Oecologia* **125**: 45–54.

Olk, D.C., Anders, M.M., Filley, T.R., Isbell, C., 2009. Crop nitrogen uptake and soil phenols accumulation under continuous rice cropping in Arkansas. *Soil Science Society of America Journal* **73**: 952.

Oremus, P.A.I., Otten, H., 1981. Factors affecting growth and nodulation of *Hippophaë rhamnoides* L. ssp. *rhamnoides* in soils from two successional stages of dune formation. *Plant and Soil* **63**: 317–331.

Otfinowski, R., 2008. Patterns and processes of exotic plant invasions in Riding Mountain National Park, Manitoba, Canada.

Owuor, P., 2016. Effects of caffeine on in vitro tea growth Effects on caffeine on in vitro tea growth. *Journal of Plantation Crops* **35**: 36.

Packer, A., Clay, K., 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature* **404**: 278–81.

Pankhurst, C.E., Blair, B.L., Magarey, R.C., Stirling, G.R., Garside, A.L., 2005. Effects of biocides and rotation breaks on soil organisms associated with the poor early growth of sugarcane in continuous monoculture. *Plant and Soil* **268**: 255–269.

Parr, J.F., Papendick, R.I., Hornick, S.B., Meyer, R.E., 1992. Soil quality: Attributes and relationship to alternative and sustainable agriculture. *American Journal of Alternative Agriculture* **7**: 5-11.

Pastor, J., Durkee Walker, R., 2006. Delays in nutrient cycling and plant population oscillations. *Oikos* **112**: 689-705.

Paterson, E., Gebbing, T., Abel, C., Sim, A., Telfer, G., 2007. Rhizodeposition shapes rhizosphere microbial community structure in organic soil. *New Phytologist* **173**: 600–610.

Patrick, Z.A., 1971. Phytotoxic substances associated with the decomposition in soil of plant residues. *Soil Science* **111**: 13–18.

Patrick, Z.A., Koch, L.W., 1958. Inhibition of respiration, germination, and growth by substances arising during the decomposition of certain plant residues in the soil. *Canadian Journal of Botany* **36**: 621–647.

Patrick, Z.A., Snyder, W.C., Toussoun, T.A., 1963. Phytotoxic substances in arable soils associated with decomposition of plant residues. *Phytopathology* **53**: 152-161

Paungfoo-Lonhienne, C., Lonhienne, T.G.A., Mudge, S.R., Schenk, P.M., Christie, M., Carroll, B.J., Schmidt S. 2010. DNA is taken up by root hairs and pollen, and stimulates root and pollen tube growth. *Plant Physiology* **153**: 799–805.

Pemadasa, M.A., 1981. Cyclic change and pattern in an *Arthrocnemum* community in Sri Lanka. *The Journal of Ecology* **69**: 565–574.

Peng, S., Bouman, B., Visperas, R.M., Castañeda, A., Nie, L., Park, H.K. 2006. Comparison between aerobic and flooded rice in the tropics: agronomic performance in an eight-season experiment. *Field Crops Research* **96**: 252-259.

Perry, L.G., Thelen, G.C., Ridenour, W.M., Weir, T.L., Callaway, R.M., Paschke, M.W., M VIVANCO, J., 2005. Dual role for an allelochemical:(±)-catechin from *Centaurea maculosa* root exudates regulates conspecific seedling establishment. *Journal of Ecology* **93**: 1126–1135.

Petermann, J.S., Fergus, A.J., Turnbull, L.A., Schmid, B. 2008. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* **89**: 2399-2406.

Pignatti, S., 1997. Ecologia del paesaggio. *UTET, Torino, Italy.*

Proebsting, E.L., Gilmore, A.E., 1941. The relation of peach root toxicity to the re-establishing of peach orchards, in: Proc. Amer. Soc. Hort. Sci. pp. 21–26.

Putnam, A.R., 1994. Phytotoxicity of plant residues. *Managing agricultural residues. Boca Raton, FL, USA: Lewis Publishers* 285–314.

Ravi, S., D'Odorico, P., Wang, L., Collins, S., 2008. Form and function of grass ring patterns in arid grasslands: the role of abiotic controls. *Oecologia* **158**: 545–555.

Reeves, D.W., 1997. The role of soil organic matter in maintaining soil quality in continuous cropping systems. *Soil and Tillage Research* **43**: 131–167.

Reinhart, K.O., Callaway, R.M., 2006. Soil biota and invasive plants. *New Phytologist* **170**: 445–457.

Reinhart, K.O., Royo, A.A., Van Der Putten, W.H., Clay, K., 2005. Soil feedback and pathogen activity in *Prunus serotina* throughout its native range. *Journal of Ecology* **93**: 890–898.

Ren, X., He, X., Zhang, Z., Yan, Z., Jin, H., Li, X., Qin, B., 2015. Isolation, identification, and autotoxicity effect of allelochemicals from rhizosphere soils of flue-cured tobacco. *Journal of Agricultural and Food Chemistry* **63**: 8975–8980.

Rice, E.L., 1984. Allelopathy, 2nd. ed. Academic Press, London.

Rumberger, A., Merwin, I.A., Thies, J.E., 2007. Microbial community development in the rhizosphere of apple trees at a replant disease site. *Soil Biology and Biochemistry* **39**: 1645–1654.

Russell, E.J., Petherbridge, F.R., 1912. Investigations on "Sickness" in Soil: II. "Sickness" in Glasshouse Soils. *The Journal of Agricultural Science* **5**: 86–111.

Rutten, G., Prati, D., Hemp, A., Fischer, M., 2016. Plant–soil feedback in East-African savanna trees. *Ecology* **97**: 294–301.

Savory, B.M., 1966. *Specific replant diseases*. Commonwealth Agricultural Bureaux; England.

Schreiner, O., Shorey, E., 1909. Isolation of harmful organic substances from soils.

Schreiner, O., Sullivan, M.X., 1908. Soil fatigue caused by organic compounds. *Journal of Biological Chemistry* **6**: 39–50.

Serracin, M., Schmitt, D., Nelson, S., 1999. Coffee Decline. *College of Tropical Agriculture and Human Resources*.

Shen, G., Lu, Y., Zhou, Q., Hong, J., 2005. Interaction of polycyclic aromatic hydrocarbons and heavy metals on soil enzyme. *Chemosphere* **61**: 1175–1182.

Shipton, P.J., 1977. Monoculture and soilborne plant pathogens. *Annual Review of Phytopathology* **15**: 387–407.

Sigüenza, C., Corkidi, L., Allen, E.B., 2006. Feedbacks of soil inoculum of mycorrhizal fungi altered by N deposition on the growth of a native shrub and an invasive annual grass. *Plant and Soil* **286**: 153–165.

Singh, H.P., Batish, D.R., Kohli, R.K., 1999. Autotoxicity: concept, organisms, and ecological significance. *Critical Reviews in Plant Sciences* **18**: 757–772.

Stark, C., Condron, L.M., Stewart, A., Di, H.J., O'Callaghan, M., 2007. Influence of organic and mineral amendments on microbial soil properties and processes. *Applied Soil Ecology* **35**: 79–93.

Stinca, A., Chirico, G.B., Incerti, G., Bonanomi, G., 2015. Regime shift by an exotic nitrogen-fixing shrub mediates plant facilitation in primary succession. *PloS one* **10**: e0123128.

Summer, D.R., Gascho, G.J., Johnson, A.W., Hook, J.E., Threadgill, E.D., 1990. Root Diseases, Populations of Soil Fungi, and Yield Decline in Continuous Double-Crop Corn. *Plant Disease*.

Tagliavini, M., Marangoni, B., 1992. Growth of peach as affected by decomposition of own root residues in soil. *Plant and Soil* **145**: 253–260.

Tang, J., Xue, Z., Daroch, M., Ma, J., 2015. Impact of continuous *Salvia miltiorrhiza* cropping on rhizosphere actinomycetes and fungi communities. *Annals of Microbiology* **65**: 1267–1275.

Teste, F.P., Kardol, P., Turner, B.L., Wardle, D.A., Zemunik, G., Renton, M., Laliberté, E., 2017. Plant-soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. *Science* **355**: 173–176.

Troelstra, S.R., Wagenaar, R., Smant, W., Peters, B.A.M. 2001. Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. *New Phytologist* **150**: 697-706.

Turkington, R.A., Cavers, P.B., Aarssen, L.W. 1977. Neighbour relationships in grass–legume communities. I. Interspecific contacts in four grassland communities near London, Ontario. *Canadian Journal of Botany* **55**: 2701-2711.

Utkhede, R.S., Smith, E.M., Palmer, R., 1992. Effect of root-rot fungi and root-lesion nematodes on the growth of young apple-trees grown in apple replant disease soil. *Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection* **99**: 414–419.

Vallance, J., Daniel, F., Floch, G. Le, Gu??rin-Dubrana, L., Blancard, D., Rey, P., 2009. Pathogenic and beneficial microorganisms in soilless cultures. *Sustainable Agriculture* **2**: 711–726.

Van de Voorde, T.F.J., Ruijten, M., van der Putten, W.H., Bezemer, T.M., 2012. Can the negative plant-soil feedback of *Jacobaea vulgaris* be explained by autotoxicity? *Basic and Applied Ecology* **13**: 533–541.

Van Der Heijden, M.G., Bardgett, R.D., Van Straalen, N.M. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* **11**: 296-310.

Van Der Putten, W.H., 1997. Die-back of *Phragmites australis* in European wetlands: An overview of the European Research Programme on Reed Die-back and Progression (1993-1994). *Aquatic Botany* **59**: 263–275.

Van der Putten, W.H., Dijk, C. Van, Peters, B. a. M., 1993. Plant-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature* **362**: 53–56.

Van der Putten, W.H., Bardgett, R.D., Bever, J.D., Bezemer, T.M., Casper, B.B., Fukami, T., Kardol, P., Klironomos, J.N., Kulmatiski, A., Schweitzer, J.A., Suding, K.N., Van de Voorde, T.F.J., Wardle, D.A., 2013. Plant-soil feedbacks: The past, the present and future challenges. *Journal of Ecology* **101**: 265–276.

Van der Putten, W.H., 2017. Belowground drivers of plant diversity. *Science* **355**: 134–135.

Venugopalan, M. V, Pundarikakshudu, R., 1999. Long-term effect of nutrient management and cropping system on cotton yield and soil fertility in rainfed vertisols **1**: 159–164.

Viator, R.P., Johnson, R.M., Grimm, C.C., Richard, E.P., 2006. Allelopathic, autotoxic, and hormetic effects of postharvest sugarcane residue. *Agronomy Journal* **98**: 1526–1531.

Vincenot, C.E., Cartenì, F., Bonanomi, G., Mazzoleni, S., Giannino, F., 2017. Plant–soil negative feedback explains vegetation dynamics and patterns at multiple scales. *Oikos* DOI: 10.1111/oik.04149

Walker, L.R., Clarkson, B.D., Silvester, W.B., Clarkson, B.R., White, P.S., 2003. Colonization dynamics and facilitative impacts of a nitrogen-fixing shrub in primary succession. *Journal of Vegetation Science* **14**: 277–290.

Waller, G.R., Anaya Lang, A.L., Sagrero-Nieves, L., Celis Ochoa Cordero, A.B., Kumari, D., Friedman, J., Friedman, N., Chou, C.H., 1990. A problem in coffee plantations: autotoxicity of

caffeine and other compounds. Association Scientifique Internationale du Café, Paris, France, pp. 363–371.

Wang, F., Wang, J., 2005. Study on autotoxic effects of aqueous extracts from eggplant residues. *Chinese Journal of Eco-Agriculture* **13**: 51-53.

Wang, M., Wu, C., Cheng, Z., Meng, H., Zhang, M., Zhang, H., 2014. Soil chemical property changes in eggplant / garlic relay intercropping systems under continuous cropping **9**: e111040

Wardle, D. a, Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. *Science* **304**: 1629–33.

Watt, A.S., 1947. Pattern and process in the plant community. *Journal of ecology* **35**: 1–22.

Webb, L.J., Tracey, J.G., Haydock, K.P., 1967. A factor toxic to seedlings of the same species associated with living roots of the non-gregarious subtropical rain forest tree *Grevillea robusta*. *Journal of applied Ecology* **4**: 13–25.

Wen, F., White, G. J., VanEtten, H.D., Xiong, Z., Hawes, M.C. 2009. Extracellular DNA is required for root tip resistance to fungal infection. *Plant Physiology* **151**: 820-829.

Westphal, A., Browne, G.T., Schneider, S., 2002. Evidence for biological nature of the grape replant problem in California. *Plant and Soil* **242**: 197–203.

Whittaker, R.H., Levin, S., 1977. The role of mosaic phenomena in natural communities. *Theoretical population biology* **12**: 117–139.

Wikberg, S., Mucina, L., Lepš, J., 2002. Spatial variation in vegetation and abiotic factors related to the occurrence of a ring-forming sedge. *Journal of vegetation Science* **13**: 677–684.

Wills, C., Condit, R., Foster, R.B., Hubbell, S.P., 1997. Strong density- and diversity-related effects help to maintain tree species diversity in a neotropical forest. *Proceedings of the National Academy of Sciences* **94**: 1252–1257.

Wilson, R.E., Rice, E.L., 1968. Allelopathy as expressed by *Helianthus annuus* and its role in old-field succession. *Bulletin of the Torrey Botanical Club* **95**: 432–448.

Wu, H., Pratley, J., Lemerle, D., An, M., Liu, D.L., 2007. Autotoxicity of wheat (*Triticum aestivum* L.) as determined by laboratory bioassays. *Plant and Soil* **296**: 85–93.

Wu, L., Chen, J., Wu, H., Wang, J., Wu, Y., Lin, S., Khan, M.U., Zhang, Z., Lin, W., 2016. Effects of consecutive monoculture of *Pseudostellaria heterophylla* on soil fungal community as determined by pyrosequencing. *Scientific reports* **6**: 26601.

Wu, Z.J., Xie, Z.K., Yang, L., Wang, R.Y., Guo, Z.H., Zhang, Y.B., Wang, L., Kutcher, H.R., 2015. Identification of autotoxins from root exudates of Lanzhou lily (*Lilium davidii* var.unicolor). *Allelopathy Journal* **35**: 35–48.

Xia, Z.C., Kong, C.H., Chen, L.C., Wang, S.L., 2015. Allelochemical-mediated soil microbial community in long-term monospecific Chinese fir forest plantations. *Applied Soil Ecology* **96**: 52–59.

Xiang, J., Haden, V.R., Peng, S., Bouman, B.A.M., Visperas, R.M., Nie, L., Huang, J., Cui, K., 2009. Improvement in nitrogen availability, nitrogen uptake and growth of aerobic rice following soil acidification. *Soil Science and Plant Nutrition* **55**: 705–714.

Xiong, W., Zhao, Q., Zhao, J., Xun, W., Li, R., Zhang, R., Wu, H., Shen, Q., 2014. Different Continuous Cropping Spans Significantly Affect Microbial Community Membership and Structure in a Vanilla-Grown Soil as Revealed by Deep Pyrosequencing. *Microbial Ecology* **70**: 209–218.

Xiong, W., Li, Z., Liu, H., Xue, C., Zhang, R., Wu, H., 2015. The effect of long-term continuous cropping of black pepper on soil bacterial communities as determined by 454 Pyrosequencing RIVISTA **10**: 1–13.

Xu, Y., Wu, Y.G., Chen, Y., Zhang, J.F., Song, X.Q., Zhu, G.P., Hu, X.W., 2015. Autotoxicity in *Pogostemon cablin* and their allelochemicals. *Brazilian Journal of Pharmacognosy* **25**: 117–123.

Yang, J. in, Ruegger, P.M., McKenry, M. V., Becker, J.O., Borneman, J., 2012. Correlations between root-associated microorganisms and peach replant disease symptoms in a California Soil. *PLoS ONE* **7**: e46420.

Yang, M., Zhang, X., Xu, Y., Mei, X., Jiang, B., Liao, J., Yin, Z., Zheng, J., Zhao, Z., Fan, L., He, X., Zhu, Y., Zhu, S., 2015. Autotoxic ginsenosides in the rhizosphere contribute to the replant failure of *Panax notoginseng*. *PLoS ONE* **10**: 1–17.

Yang, R.X., Gao, Z.G., Liu, X., Yao, Y., Cheng, Y., 2014. Root exudates from muskmelon (*Cucumis melon*. L) induce autotoxicity and promote growth of *Fusarium oxysporum* f. sp. *melonis*. *Allelopathy Journal* **33**: 175–187.

Ye, S.F., Yu, J.Q., Peng, Y.H., Zheng, J.H., Zou, L.., 2004. Incidence of Fusarium wilt in *Cucumis sativus* L s promoted by cinnamic acid, an autotoxin in root exudates. *Plant Soil,* **263**: 143–150.

Yu, J.Q., Lee, K.S., Matsui, Y., 1993. Effect of the addition of activated charcoal to the nutrient solution on the growth of tomato in hydroponic culture. *Soil Science and Plant Nutrition* **39**: 13–22.

Yu, J.Q., Matsui, Y., 1993. Extraction and identification of phytotoxic substances accumulated in nutrient solution for the hydroponic culture of tomato. *Soil Science and Plant Nutrition* **39**: 691–700.

Yu, J.Q., Matsui, Y., 1994. Phytotoxic Substances in Root Exudates of Cucumber (*Cucumis sativus* L.). *Journal of Chemical Ecology* **20**: 21–31.

Yu, J.Q., Shou, S.Y., Qian, Y.R., Zhu, J.Z., Hu, W.H., 2000. Autotoxic potential of cucurbit crops. *Plant and Soil* **223**: 147–151.

Yu, J.Q., 2001. Autotoxic potential of cucurbit crops: phenomenon, chemicals, mechanisms and means to overcome. *Journal of crop production* **4**: 335–348.

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., Tilman, D., 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology* **84**: 2042–2050.

Zhang, Q., 1993. Potential role of allelopathy in the soil and the decomposing root of Chinese-fir replant woodland. *Plant and Soil* **151**: 205–210.

Zhang, X.H., Zhang, E.H., Fu, X.Y., Huang, Y., Lang, D.Y., 2010a. Autotoxic effects of *Angelica sinensis* (Oliv.) Diels. *Allelopathy Journal* **26**: 1–12.

Zhang, S., Jin, Y., Zhu, W., Tang, J., Hu, S., Zhou, T., Chen, X., 2010b. Baicalin released from *Scutellaria baicalensis* induces autotoxicity and promotes soilborne pathogens. *Journal of Chemical Ecology* **36**: 329–338.

Zhang, X.-H., Zhang, E.-H., Lang, D.-Y., 2011. Autotoxic compounds from rhizosphere soil of *Humulus lupulus* L. extracts: identification and biological activity. *Agronomy Journal* **103**: 695–701.

Zhang, X., Lang, D., Zhang, E., Wang, Z., 2015. Effect of autotoxicity and soil microbes in continuous cropping soil on *Angelica sinensis* seedling growth and rhizosphere soil microbial population. *Chinese Herbal Medicines* **7**: 88–93.

Zhou, K., Zhou, X., Yang, L., Hao, F., Wang, Z., Guo, W., 2009. Autotoxic effects of Chrysanthemum (*Chrysanthemum morifolium* (Ramat) Tzvel.). *Allelopathy Journal* **24**: 91-101.

Zhou, X., Wu, F., 2012. Dynamics of the diversity of fungal and Fusarium communities during continuous cropping of cucumber in the greenhouse. *FEMS Microbiology Ecology* **80**: 469–478.

Zhou, X., Gao, D., Liu, J., Qiao, P., Zhou, X., Lu, H., Wu, X., Liu, D., Jin, X., Wu, F., 2014. Changes in rhizosphere soil microbial communities in a continuously monocropped cucumber (*Cucumis sativus* L.) system. *European Journal of Soil Biology* **60**: 1–8.

Zhou, X., Wu, F., 2015. Changes in soil chemical characters and enzyme activities during continuous monocropping of cucumber (Cucumis sativus). *Pakistan Journal of Botany* **47**: 691–697.

Zucconi, F., 2003. Declino del suolo e stanchezza del terreno. Pitagora.

Figures and tables

Fig. 1. Number of papers published in the last 117 years on soil sickness in agro-ecosystems, and negative plant-soil feedback in natural plant communities (data from Scopus accessed on March, 2017).

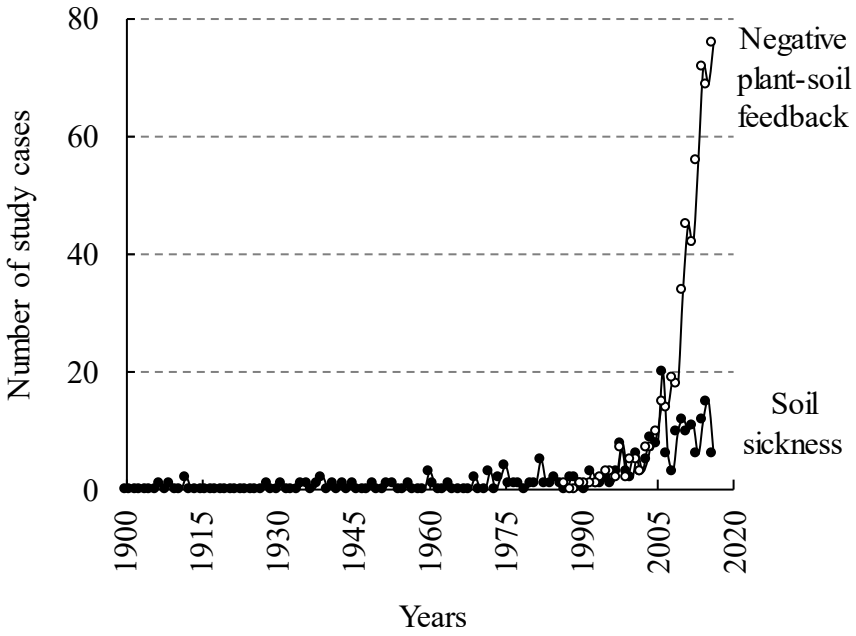

Fig. 2. Relative occurrence of soil sickness cases (complete list reported in table 1) for plant families (a), life forms (b) and climate zones (c).

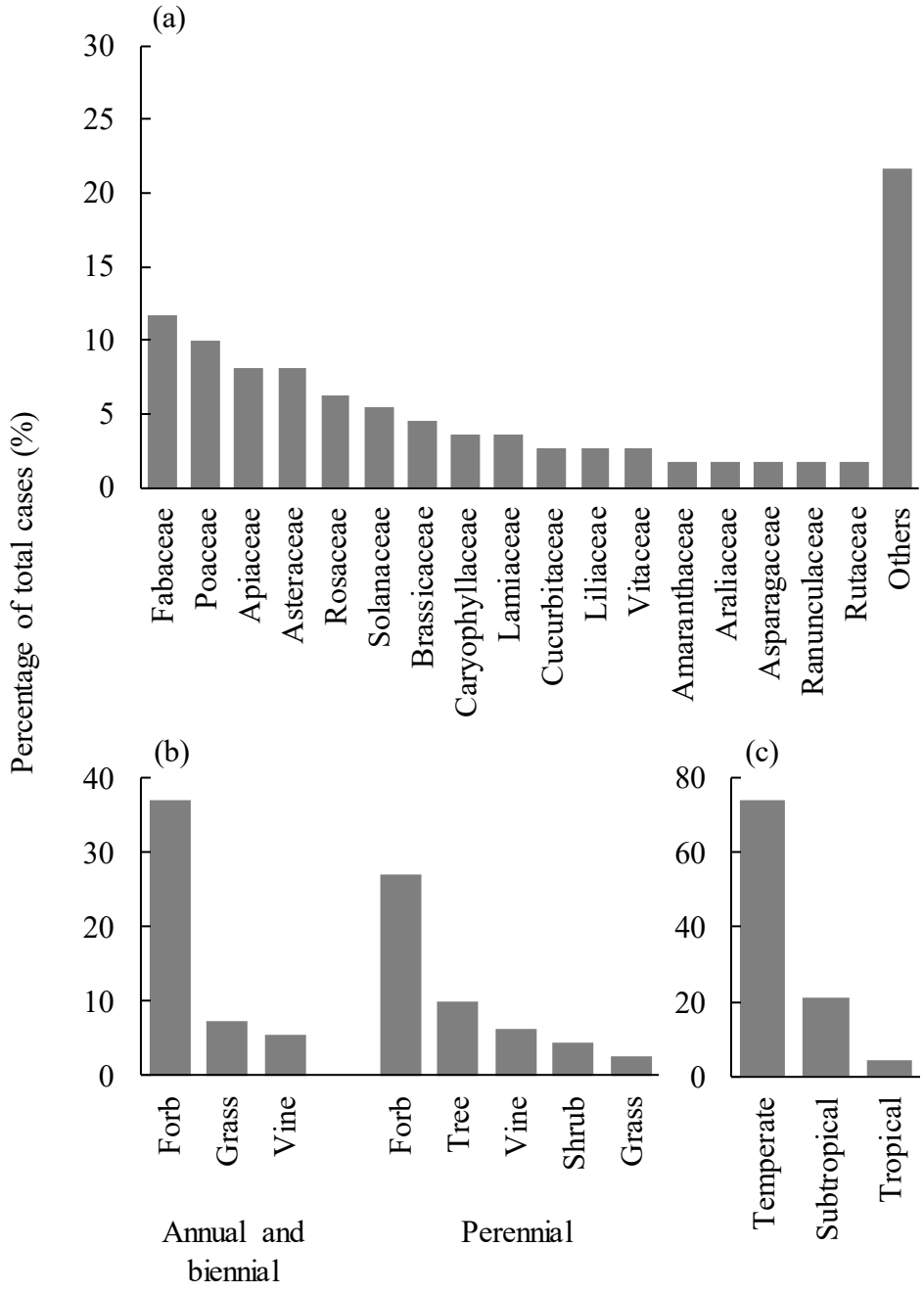

Fig. 3. Relative occurrence of negative plant-soil feedback cases (complete list reported in Annex table 1) for plant families (a), life forms (b), and climate zones (c).

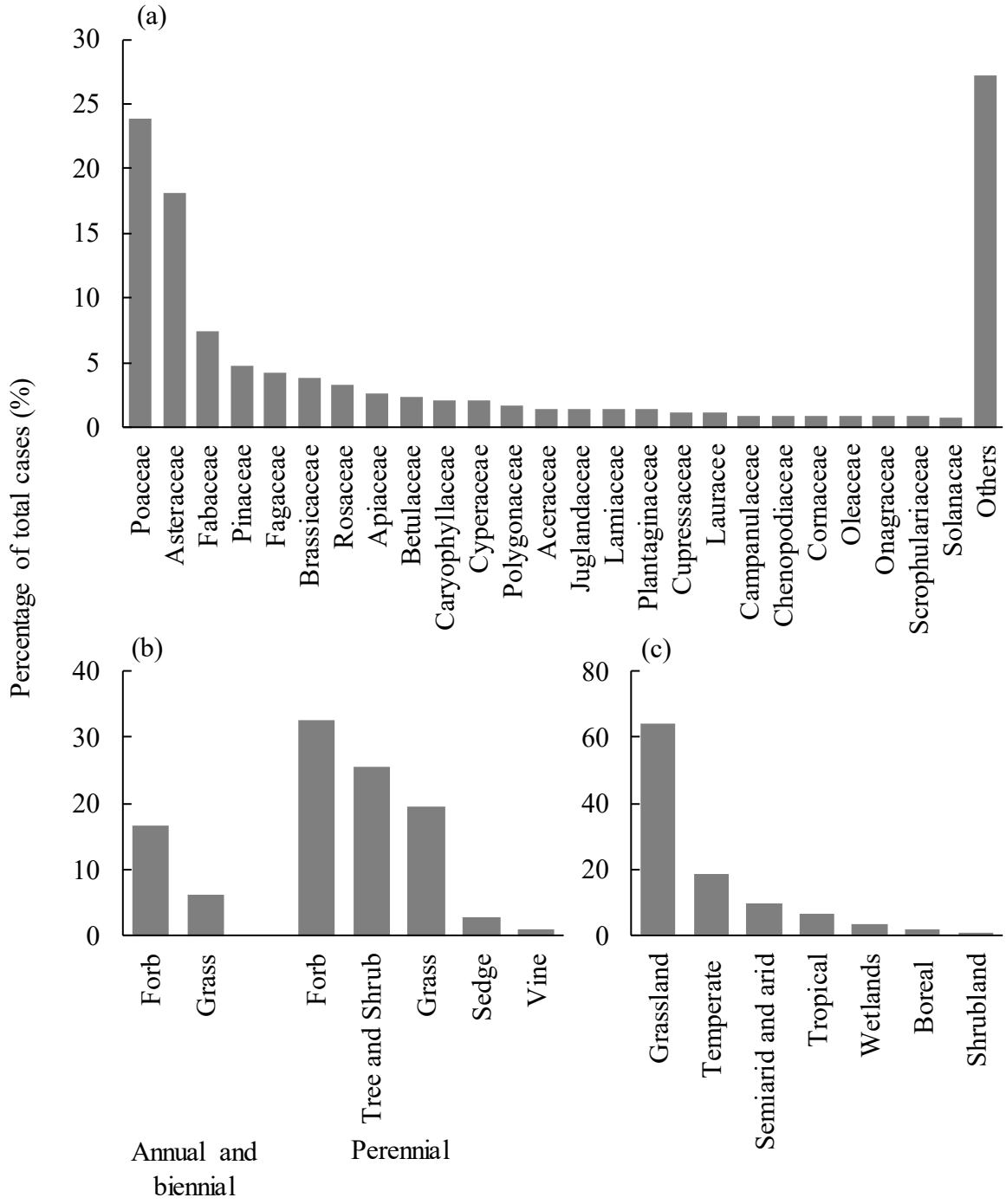

Fig. 4. Examples of soil sickness in agro-ecosystems and plant soil feedback in natural ecosystems. (A) Fourth range lettuce cultivation showing extensive damping-off symptoms; (B) Seedling failure under conspecific tree resulting in the Janzen-Connell recruitment distribution in natural forest; (C) Examples of spot and "ring" formed by perennial grasses capable of clonal propagation (pictures taken by Giuliano Bonanomi).

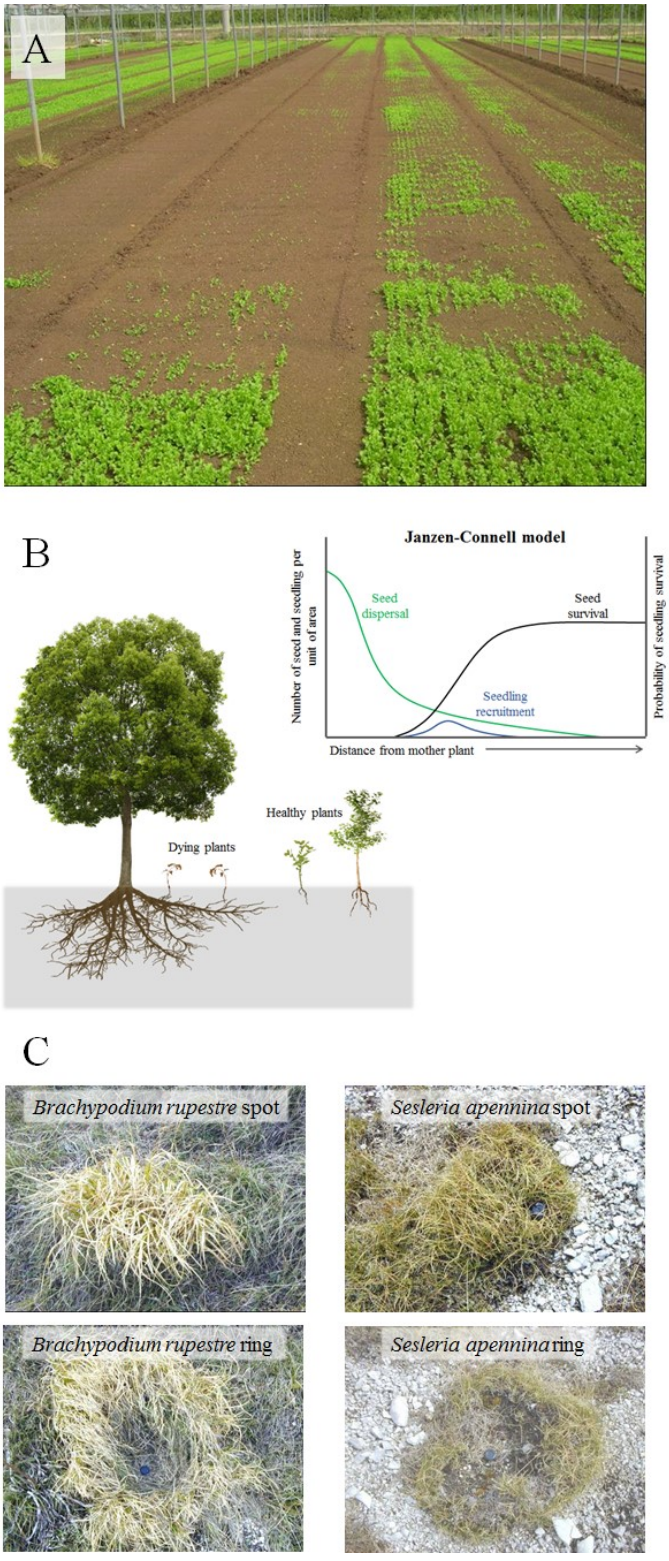

Fig. 5. Schematic representation of autotoxicity effects associated with the decomposition of crop residues, including nutrient immobilization due to microbial competition, release of low-molecular weight phytotoxic compounds and extracellular DNA from conspecific plants tissues.

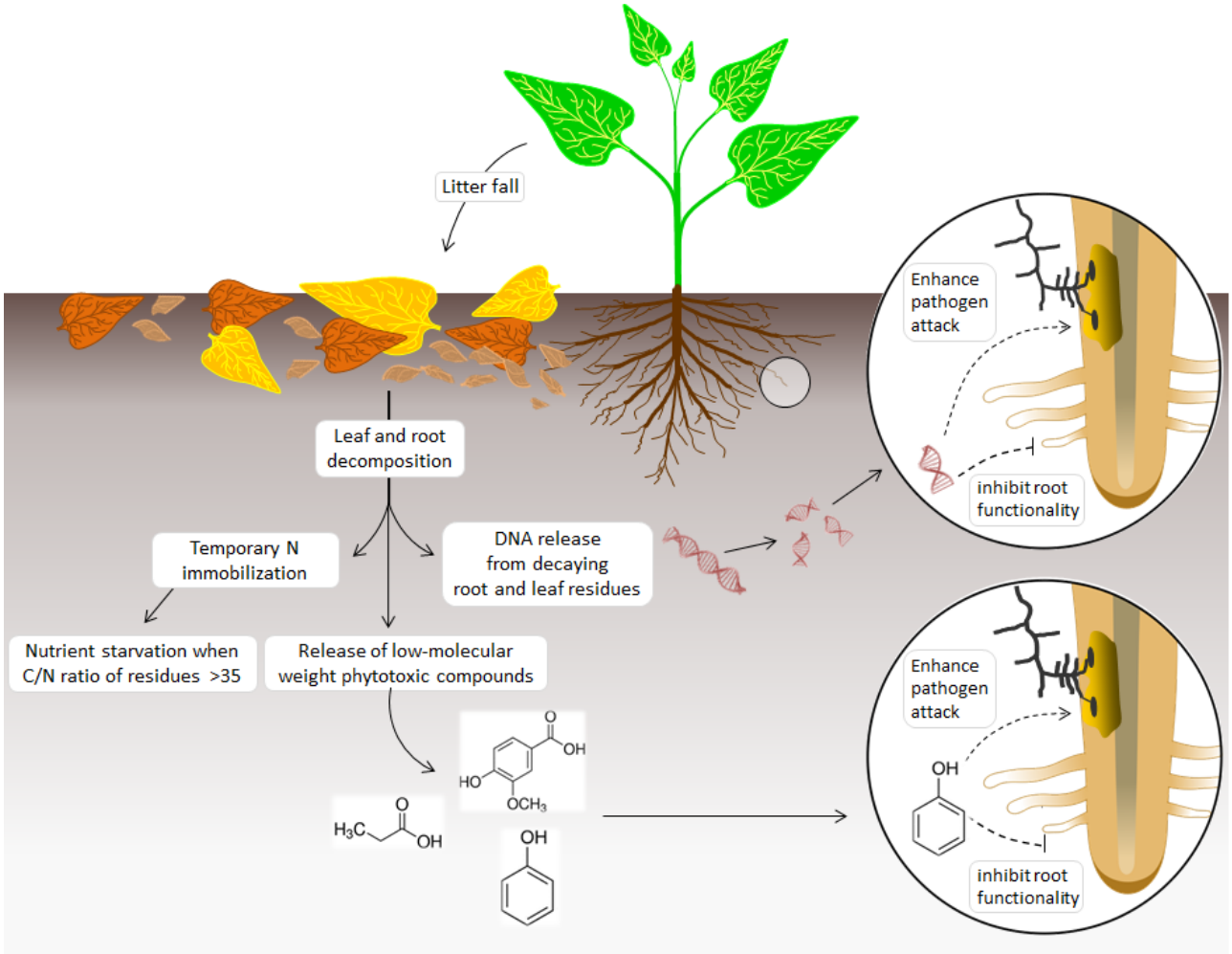

Table 1. List of experimental studies reporting soil sickness in agro-ecosystems. Taxonomic family, life form and life cycle, experimental conditions and the putative mechanism/s causing soil sickness as proposed by the authors are reported. Studies are ordered alphabetically by plant name.

N°	Species	Family	Life form	Life cycle	Ecosystem	Experimental condition	Putative mechanisms	References
1	*Agrostemma githago*	Caryophyllaceae	Forb	Annual	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
2	*Ammi majus*	Apiaceae	Forb	Annual	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
3	*Angelica sinensis*	Apiaceae	Forb	Perennial	Temperate	Field – Pot	Autotoxicity and soilborne pathogens	Zhang et al., 2015
					Temperate	Growth chamber – *In vitro* and pot	Autotoxicity	Zhang et al., 2010a
4	*Antirrhinum majus*	Plantaginaceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
5	*Apium graveolens*	Apiaceae	Forb	Perennial	Temperate	Growth chamber – *In vitro*	Autotoxicity	Asao et al., 2004
6	*Aquilegia flabellata*	Ranunculaceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
7	*Arachis hypogaea*	Fabaceae	Forb	Annual	Temperate	Growth chamber – *In vitro*	Autotoxicity	Huang et al., 2013
					Temperate	Growth chamber – Pot	Microbial shift and soilborne pathogens	Li et al., 2014a
					Temperate		Autotoxicity and soilborne pathogens	Li et al., 2010a
8	*Asparagus officinalis*	Asparagaceae	Forb	Perennial	Temperate	Growth chamber – *In vitro* / Greenhouse – Pot	Autotoxicity	Miller et al., 1991
					Subtropical	Field – Plot	Soilborne pathogens	Nigh, 1990
					Temperate	Growth chamber – *In vitro* / Greenhouse – Pot	Autotoxicity and soilborne pathogens	Hartung and Stephens, 1983
9	*Avena sativa*	Poaceae	Grass	Annual	Temperate	Growth chamber – Pot	Autotoxicity	Nielsen et al., 1960

10	*Beta vulgaris*	Chenopodiaceae	Forb	Biennial	Temperate	Field – Pot	Soilborne pathogens and nematodes	Crump and Kerry, 1987
11	*Brassica campestris*	Brassicaceae	Forb	Biennial	Temperate	Growth chamber – *In vitro*	Autotoxicity	Asao et al., 2004
12	*Brassica napus*	Brassicaceae	Forb	Annual	Temperate	Greenhouse – Pot / Field – Plot	Microbial shift and soilborne pathogens	Hilton et al., 2013
13	*Brassica oleracea*	Brassicaceae	Forb	Biennial	Temperate	Growth chamber – *In vitro*	Autotoxicity	Asao et al., 2004
14	*Brassica rapa*	Brassicaceae	Forb	Biennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
15	*Calendula officinalis*	Asteraceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
16	*Callistephus chinensis*	Asteraceae	Forb	Annual	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
17	*Camellia sinensis*	Theaceae	Shrub	Perennial	Tropical	Growth chamber – *In vitro*	Autotoxicity	Owuor, 2016
18	*Capsicum annuum*	Solanaceae	Forb	Perennial	Temperate	Greenhouse – Plot	Soilborne pathogens	Martínez et al., 2011
19	*Carthamus tinctorius*	Asteraceae	Forb	Annual	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
20	*Carum copticum*	Apiaceae	Forb	Annual	Subtropical	Growth chamber – *In vitro*	Autotoxicity	Chaturvedi and Muralia, 1975
21	*Celosia argentea*	Amaranthaceae	Forb	Annual	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
22	*Chrysanthemum coronarium*	Asteraceae	Forb	Annual	Temperate	Growth chamber – *In vitro*	Autotoxicity	Asao et al., 2004
23	*Chrysanthemum morifolium*	Asteraceae	Forb	Annual	Temperate		Autotoxicity	Zhou et al., 2009
24	*Cicer arietinum*	Fabaceae	Forb	Annual			Not reported	Rice, 1984
25	*Citrullus lanatus*	Cucurbitaceae	Annual vine	Annual	Temperate	Growth chamber – *In vitro* / Greenhouse – Hydroponic	Autotoxicity	Hao et al., 2007
					Temperate	Growth chamber – *In vitro* and pot	Autotoxicity	Yu et al., 2000

26	*Citrus aurantium*	Rutaceae	Tree	Perennial	Subtropical	Growth chamber – *In vitro* / Greenhouse – Pot	Autotoxicity and soilborne pathogens	Hassan et al., 1989
27	*Citrus jambhiri*	Rutaceae	Tree	Perennial	Subtropical	Greenhouse – Hydroponic	Autotoxicity	Burger and Small, 1983
28	*Coffea arabica*	Rubiaceae	Shrub	Perennial	Tropical	Growth chamber – *In vitro*	Autotoxicity	Waller et al., 1990
					Tropical		Nematodes	Serracin et al., 1999
29	*Colocasia esculenta*	Araceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic	Autotoxicity	Asao et al., 2003
30	*Coriandrum sativum*	Apiaceae	Forb	Annual	Subtropical	Growth chamber – *In vitro*	Autotoxicity	Chaturvedi and Muralia, 1975
31	*Crocus sativus*	Iridaceae	Forb	Perennial	Temperate	Field – Plot	Not reported	Gresta et al., 2016
32	*Cucumis melo*	Cucurbitaceae	Annual vine	Annual	Temperate	Growth chamber – *In vitro* and pot	Autotoxicity	Yu et al., 2000
					Temperate	Growth chamber – *In vitro* and pot / Field – Plot	Autotoxicity and soilborne pathogens	Yang et al., 2014
33	*Cucumis sativus*	Cucurbitaceae	Annual vine	Annual	Temperate	Greenhouse – Hydroponic	Autotoxicity	Yu and Matsui, 1994
					Temperate	Greenhouse – Pot	Soilborne pathogens	Zhou and Wu, 2012
					Temperate	Greenhouse – Hydroponic	Autotoxicity and soilborne pathogens	Ye et al., 2004
					Temperate	Greenhouse – Pot	Microbial shift	Zhou et al., 2014
34	*Cuminum cyminum*	Apiaceae	Forb	Annual	Subtropical	Growth chamber – *In vitro*	Autotoxicity	Chaturvedi and Muralia, 1975
35	*Daucus carota*	Apiaceae	Forb	Biennial	Subtropical	Growth chamber – *In vitro*	Autotoxicity	Chaturvedi and Muralia, 1975
36	*Delphinium ajacis*	Ranunculaceae	Forb	Annual	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
37	*Dianthus caryophyllus*	Caryophyllaceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007

38	*Eustoma grandiflorum*	Gentianaceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
39	*Ficus carica*	Moraceae	Tree	Perennial	Temperate	Greenhouse – Pot	Soilborne pathogens	Hosomi and Uchiyama, 1998
40	*Foeniculum vulgare*	Apiaceae	Forb	Annual	Subtropical	Growth chamber – *In vitro*	Autotoxicity	Chaturvedi and Muralia, 1975
41	*Fragaria x ananassa*	Rosaceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic	Autotoxicity	Asaduzzaman et al., 2012
42	*Glycine max*	Fabaceae	Forb	Annual	Temperate		Autotoxicity	Han et al., 2002
					Temperate	Field – Plot	Microbial shift	Li et al., 2010b
43	*Godetia amoena*	Onagraceae	Forb	Annual	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
44	*Gomphrena globosa*	Amaranthaceae	Forb	Annual	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
45	*Gossypium* spp.	Malvaceae	Shrub	Perennial	Temperate		Autotoxicity	Jiang et al., 2013
					Temperate	Growth chamber – *In vitro* / Greenhouse – Pot	Microbial shift and soilborne pathogens	Li et al., 2015
46	*Gypsophila elegans*	Caryophyllaceae	Forb	Annual	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
47	*Helianthus annuus*	Asteraceae	Forb	Annual	Subtropical	Greenhouse – Pot	Autotoxicity	Wilson and Rice, 1968
48	*Hordeum vulgare*	Poaceae	Grass	Annual	Subtropical	Growth chamber – *In vitro*	Autotoxicity	Ben-Hammouda et al., 2002
					Temperate	Greenhouse – Pot	Microbial shift	Alström, 1992
					Temperate	Field – Plot	Soilborne pathogens	Delogu et al., 2003
49	*Humulus lupulus*	Cannabaceaae	Perennial vine	Perennial	Temperate	Greenhouse – Pot	Autotoxicity	Zhang et al., 2011
50	*Ipomea batatas*	Convolvulaceae	Perennial vine	Perennial	Tropical	Field – Plot	Nematodes	Hartemink et al., 2000

51	*Juglans nigra*	Juglandaceae	Tree	Perennial			Soilborne pathogens	Grente, 1963
52	*Lactuca sativa*	Asteraceae	Forb	Annual	Temperate	Growth chamber – *In vitro*	Autotoxicity	Asao et al., 2004
53	*Lathyrus odoratus*	Fabaceae	Annual vine	Annual	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
54	*Lilium davidii*	Liliaceae	Forb	Perennial	Temperate	Growth chamber – *In vitro*	Autotoxicity	Wu et al., 2015
55	*Lilium x elegans*	Liliaceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
56	*Lilium x formolongi*	Liliaceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
57	*Limonium sinuatum*	Plumbaginaceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
58	*Linum usitatissimum*	Linaceae	Forb	Annual			Autotoxicity	Börner, 1960
59	*Lolium rigidum*	Poaceae	Grass	Annual	Temperate	Growth chamber – *In vitro* / Greenhouse – Pot	Autotoxicity	Canals et al., 2005
60	*Malus domestica*	Rosaceae	Tree	Perennial	Subtropical	Field – Plot	Microbial shift	Rumberger et al., 2007
					Temperate	Greenhouse – Pot	Soilborne pathogens and nematodes	Utkhede et al., 1992
					Subtropical	Field – Plot	Soilborne pathogens	Mazzola, 1998
61	*Malus* spp.	Rosaceae	Tree	Perennial			Autotoxicity	Börner, 1959 in Singh et al., 1999
62	*Manihot esculenta*	Euphorbiaceae	Forb	Perennial	Subtropical	Field – Plot	Nutrient imbalance or depletion	Howeler and Cadavid, 1990
63	*Matthiola incana*	Brassicaceae	Forb	Annual	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
64	*Medicago sativa*	Fabaceae	Forb	Perennial	Temperate	Growth chamber – *In vitro*	Autotoxicity	Chon et al., 2002
					Temperate	Growth chamber – *In vitro*	Autotoxicity and soilborne pathogens	Bonanomi et al., 2011

65	*Musa* sp.	Musaceae	Forb	Perennial	Tropical	Field – Plot	Nutrient imbalance or depletion	Bekunda, 1999
66	*Narcissus tazetta*	Amaryllidaceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
67	*Nicotiana tabacum*	Solanaceae	Forb	Annual	Subtropical	Growth chamber – *In vitro*	Autotoxicity	Ren et al., 2015
68	*Ocimum basilicum*	Lamiaceae	Forb	Annual	Temperate	Greenhouse – Plot	Not reported	Minuto et al., 2002
69	*Olea europaea*	Oleaceae	Tree	Perennial	Temperate	Greenhouse – Pot	Autotoxicity	Endeshaw et al., 2015
70	*Oryza sativa*	Poaceae	Grass	Annual	Subtropical	Field – Plot	Nutrient imbalance or depletion	Olk et al., 2009
					Subtropical	Growth chamber – *In vitro* / Field – Plot	Autotoxicity	Chou and Lin, 1976
					Subtropical	Greenhouse – Pot	Nematodes and nutrient imbalance or depletion	Kreye et al., 2009
					Subtropical	Greenhouse – Pot	Nutrient imbalance or depletion	Nie et al., 2009
71	*Panax notoginseng*	Araliaceae	Forb	Perennial	Subtropical	Growth chamber – *In vitro* / Greenhouse – Hydroponic	Autotoxicity	Yang et al., 2015
72	*Panax quinquefolium*	Araliaceae	Forb	Perennial	Temperate	Growth chamber – In vitro	Autotoxicity	He et al., 2009
73	*Papaver rhoeas*	Papaveraceae	Forb	Annual	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
74	*Parthenium argentatum*	Asteraceae	Shrub	Perennial	Subtropical	Greenhouse – Pot	Autotoxicity	Bonner and Galston, 1944
75	*Petroselinum crispum*	Apiaceae	Forb	Biennial	Temperate	Growth chamber – *In vitro*	Autotoxicity	Asao et al., 2004
76	*Phaseolus vulgaris*	Fabaceae	Forb	Annual	Temperate	Growth chamber – *In vitro* / Greenhouse – Hydroponic	Autotoxicity	Asaduzzaman and Asao, 2012
77	*Phleum pratense*	Poaceae	Grass	Perennial	Temperate	Growth chamber – Pot	Autotoxicity	Nielsen et al., 1960

78	*Physalis alkekengi*	Solanaceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
79	*Piper nigrum*	Piperaceae	Perennial vine	Perennial	Subtropical	Greenhouse – Pot	Microbial shift	Xiong et al., 2015
80	*Pisum sativum*	Fabaceae	Annual vine	Annual	Temperate	Growth chamber – *In vitro* / Greenhouse – Hydroponic	Autotoxicity	Asaduzzaman and Asao, 2012
					Temperate	Greenhouse – Pot	Soilborne pathogens	Bodker and Leroul, 1993
					Temperate	Field – Plot	Microbial shift	Nayyar et al., 2009
81	*Platycodon grandiflorum*	Campanulaceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
82	*Pogostemon cablin*	Lamiaceae	Forb	Annual	Subtropical	Greenhouse – Hydroponic / Field – Pot	Autotoxicity	Xu et al., 2015
83	*Prunus avium*	Rosaceae	Tree	Perennial	Temperate	Greenhouse – Pot	Soilborne pathogens	Hoestra, 1965
84	*Prunus dulcis*	Rosaceae	Tree	Perennial	Subtropical	Greenhouse – Pot	Soilborne pathogens	Fatemi, 1980
85	*Prunus persica*	Rosaceae	Tree	Perennial	Temperate	Greenhouse – Pot	Autotoxicity	Tagliavini and Marangoni, 1992
					Subtropical	Greenhouse – Pot	Soilborne pathogens	Yang et al., 2012
					Temperate	Growth chamber – Pot	Microbial shift	Benizri et al., 2005
86	*Prunus serotina*	Rosaceae	Tree	Perennial	Temperate	Field – Pot	Soilborne pathogens	Reinhart et al., 2005
87	*Pseudostellaria heterophylla*	Caryophyllaceae	Forb	Perennial	Subtropical	Field – Plot	Microbial shift	Wu et al., 2016
88	*Rehmannia glutinosa*	Orobanchaceae	Forb	Perennial	Temperate	Growth chamber – *In vitro*	Soilborne pathogens	Bu et al., 2014
					Temperate	Growth chamber – *In vitro*	Autotoxicity	Li et al., 2012
89	*Rudbeckia hirta*	Asteraceae	Forb	Biennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
90	*Saccharum officinarum*	Poaceae	Grass	Perennial	Tropical	Greenhouse – Pot / Field – Plot	Soilborne pathogens and nematodes	Pankhurst et al., 2005

91	*Saccharum* spp.	Poaceae	Grass	Perennial	Subtropical	Greenhouse – Pot	Autotoxicity	Viator et al., 2006
92	*Salvia miltiorrhiza*	Lamiaceae	Shrub	Perennial	Subtropical	Field – Plot	Microbial shift	Tang et al., 2015
93	*Scutellaria baicalensis*	Lamiaceae	Forb	Perennial	Temperate	Growth chamber – *In vitro* and pot	Autotoxicity and soilborne pathogens	Zhang et al., 2010b
94	*Setaria italica*	Poaceae	Grass	Annual			Autotoxicity	Lee et al., 1967 in Singh et al., 1999
95	*Solanum lycopersicum*	Solanaceae	Forb	Annual	Temperate	Greenhouse – Plot	Microbial shift	Li et al., 2014b
					Temperate	Growth chamber – *In vitro* and hydroponic	Autotoxicity	Yu and Matsui, 1993
					Temperate	Growth chamber – *In vitro* / Greenhouse – Pot	Autotoxicity and soilborne pathogens	Bonanomi et al., 2007
96	*Solanum melongena*	Solanaceae	Forb	Annual	Temperate	Growth chamber – *In vitro*	Autotoxicity	Wang and Wang, 2005
97	*Solanum tuberosum*	Solanaceae	Forb	Perennial	Temperate	Field – Plot	Microbial shift	Larkin and Honeycutt, 2006
98	*Sorghum bicolor*	Poaceae	Grass	Annual			Not reported	Rice, 1984
99	*Trifolium alexandrinum*	Fabaceae	Forb	Annual	Temperate	Field – Pot	Nutrient imbalance or depletion	Katznelson, 1972
100	*Trifolium pratense*	Fabaceae	Forb	Perennial	Temperate	Growth chamber – *In vitro* and pot	Autotoxicity	Chang et al., 1969
101	*Trifolium resupinatum*	Fabaceae	Forb	Annual	Temperate	Field – Pot	Nutrient imbalance or depletion	Katznelson, 1972
102	*Triteleia laxa*	Asparagaceae	Forb	Perennial	Temperate	Greenhouse – Hydroponic and pot	Autotoxicity	Asao et al., 2007
103	*Triticum aestivum*	Poaceae	Grass	Annual	Temperate	Growth chamber – *In vitro*	Autotoxicity	Wu et al., 2007
104	*Vanilla planifolia*	Orchidaceae	Perennial vine	Perennial	Subtropical	Greenhouse – Pot	Microbial shift and soilborne pathogens	Xiong et al., 2014
105	*Vicia faba*	Fabaceae	Forb	Annual	Temperate	Growth chamber – *In vitro* / Greenhouse – Hydroponic	Autotoxicity	Asaduzzaman and Asao, 2012

106	*Vigna radiata*	Fabaceae	Annual vine	Annual			Autotoxicity	Tang and Zhang 1986 in Singh et al., 1999
107	*Vigna unguiculata*	Fabaceae	Forb	Annual	Temperate	Growth chamber – *In vitro*	Autotoxicity	Huang et al., 2010
108	*Vitis riparia*	Vitaceae	Perennial vine	Perennial			Autotoxicity	Brinker and Creasy 1988 in Singh et al., 1999
109	*Vitis rupestris*	Vitaceae	Perennial vine	Perennial			Autotoxicity	Brinker and Creasy 1988 in Singh et al., 1999
110	*Vitis vinifera*	Vitaceae	Perennial vine	Perennial	Temperate	Greenhouse – Pot	Soilborne pathogens and nematodes	Westphal et al., 2002
111	*Zea mays*	Poaceae	Grass	Annual	Temperate	Growth chamber – *In vitro*	Autotoxicity	Martin et al., 1990
					Temperate	Field – Plot	Nutrient imbalance or depletion	Gentry et al., 2013
					Temperate	Field – Plot	Soilborne pathogens	Summer et al., 1990

Table 2. List of experimental studies reporting a promotion of soilborne pathogens activity by autotoxic factors released by roots during exudation, or during decomposing of plant residues.

Species	Autotoxic compounds	Microorganisms	References
Angelica sinensis	Root exudates	Microbial shift	Zhang et al., 2015
Arachis hypogaea	P-hydroxybenzoic acid, vanillic acid and coumalic acid	*Fusarium solani*	Li et al., 2010a
Asparagus officinalis	Root and rhizome tissues	*Fusarium oxysporum* f. sp. *asparagi* and *Fusarium moniliforme*	Hartung and Stephens, 1983
Citrus aurantium	Root residues	*Phytophthora citrophthora, Pythium aphanidermatum* and *Fusarium solani*	Hassan et al., 1989
Cucumis melo	Gallic acid, phthalic acid, syringic acid, salicylic acid, ferulic acid, benzoic acid and cinnamic acid	*Fusarium oxysporum* f. sp. *melonis*	Yang et al., 2014
Cucumis sativus	Cinnamic acid	*Fusarium oxysporum* f. sp. *cucumerinum*	Ye et al., 2004
Medicago sativa	Leaves residues	*Pythium ultimum and Rhizoctonia solani*	Bonanomi et al., 2011
Pseudostellaria heterophylla	Root exudates	*Talaromyces helicus, Kosakonia sacchari*	Wu et al., 2016
Scutellaria baicalensis	flavone baicalin (7-glucuronic acid, 5, 6-dihydroxy-flavone	*Pythium ultimum* and *Rhizoctonia solani*	Zhang et al., 2010b
Solanum lycopersicum	Leaves and roots residues	*Fusarium oxysporum* f.sp. *lycopersici*	Bonanomi et al., 2007

Table 3. Comparison between management strategies aimed at overcoming soil sickness in agro-ecosystems, and plant behavior evolved in natural ecosystems to avoid NPSF.

Agro-ecosystem	Natural ecosystem
Crop rotation (Gresta et al., 2016; Huang et al., 2013)	- Alternation of trees species in temperate (Fox 1977; Whittaker and Levin, 1977) and tropical forests (Augspurger, 1984; Mangan et al., 2010) - Cyclic succession in heathland and shrubland (Watt 1947, Bonanomi et al., 2005a) - "Carousel" dynamics in grassland dominated by short-lived plants (Maarel and Sykes, 1993; Vincenot et al., 2017)
Polyculture (Matson et al., 1997) and soil amendment with organic amendment (Bulluck et al., 2002; Stark et al., 2007)	- Litter "mixing" effect and "herd" immunity hypothesis (Hättenschwiler et al., 2005; Wills et al. 1997)
Replacing sick soil (Zucconi, 2003)	- Soil accretion in sand dune (Van der Putten et al., 1993) and river banks (Bonanomi et al., 2014)
Use of activated carbon in field conditions and in soilless systems (Elmer and Pignatello, 2011; Yu et al., 1993)	- "*Terra preta*" soil rich of charred organic materials in semi-natural ecosystems (DeLuca et al., 2006; Glaser and Birk, 2012; Kammann et al., 2016)
Soil flooding (Newhall, 1955; Nie et al., 2009)	- Lack of NPSF in aquatic environments (Bonanomi et al., 2011)

Annex table 1. List of experimental studies reporting negative plant-soil feedback (NPSF) in natural ecosystems. Taxonomic family, life form and life cycle, experimental conditions and the putative mechanism/s causing soil sickness as proposed by the authors are reported. Studies are ordered alphabetically by plant name

N°	Species	Family	Life form	Life cycle	Ecosystem	Putative mechanisms	References
1	*Abies balsamea*	Pinaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
2	*Acacia koa*	Fabaceae	Tree/Shrub	Perennial	Grassland	Microbial shift	Klironomos 2002
3	*Acacia nilotica*	Fabaceae	Tree/Shrub	Perennial	Semiarid/arid	Microbial shift	Rutten et al., 2016
4	*Acanthus mollis*	Acanthaceae	Forb	Perennial	Temperate forest	Autotoxicity	Mazzoleni et al., 2015
5	*Acer negundo*	Aceraceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Reinhart and Callaway 2004
6	*Acer platanoides*	Aceraceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Reinhart et al., 2005
7	*Acer rubrum*	Aceraceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Nijjer et al., 2007, McCarthyNeumann and Kobe 2010a, Bennett et al., 2017
8	*Acer saccharinum*	Aceraceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
9	*Acer saccharum*	Aceraceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	McCarthy Neumann and Kobe 2010a, Kotanen 2007, Bennett et al., 2017
10	*Achillea millefolium*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Schittko et al., 2016, Maron et al., 2016, Klironomos 2002, Cortois et al., 2016, Bezemer et al., 2006a, Anacker et al., 2014
11	*Acomastylis rossii*	Rosaceae	Forb	Perennial	Grassland	Microbial shift	Suding et al., 2004
12	*Acroptilon repens*	Asteraceae	Forb	Perennial	Grassland	Autotoxicity	Morris et al., 2006
13	*Aegilops triuncialis*	Poaceae	Grass	Annual	Grassland	Microbial shift	Batten et al., 2008
14	*Agalinis gattingeri*	Orobanchaceae	Forb	Annual	Grassland	Microbial shift	Klironomos 2002
15	*Ageratina adenophora*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Niu et al., 2007

16	*Agropyron cristatum*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Jordan et al., 2008
17	*Agropyron repens*	Poaceae	Grass	Perennial	Semiarid/arid	Autotoxicity	Bokhari 1978
18	*Agrostis capillaris*	Poaceae	Grass	Perennial	Grassland	Microbial shift/Soilborne pathogen/Nutrient imbalance or depletion	Zhang et al., 2016, Wubs and Bezemer 2016, Jing et al., 2015, De Deyn et al., 2004, Bezemer et al., 2006a, Bezemer et al., 2006b
19	*Agrostis gigantea*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
20	*Agrostis scabra*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
21	*Agrostis tenuis*	Poaceae	Grass	Perennial	Wetlands	Autotoxicity	Wedin and Tilman 1993, Tilman and Wedin 1991
22	*Aletris farinosa*	Nartheciaceae	Forb	Perennial	Grassland	Microbial shift	Bennett et al., 2017
23	*Alnus incana*	Betulaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Chapin et al., 1994
24	*Alnus sinuata*	Betulaceae	Tree/Shrub	Perennial	Boreal forest	Not reported	Kardol et al., 2007
25	*Alopecurus geniculatus*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Bradow and Connick 1988
26	*Amaranthus palmeri*	Amaranthaceae	Forb	Annual	Semiarid/arid	Autotoxicity	Anaya and Amo 1978
27	*Ambrosia cumanensis*	Asteraceae	Forb	Annual	Grassland	Autotoxicity	Lou et al., 2014
28	*Ambrosia trifida*	Asteraceae	Forb	Annual	Grassland	Microbial shift	Friedman et al., 1982
29	*Ammi majus*	Apiaceae	Forb	Biennial	Tropical/subtropical forest	Autotoxicity	
30	*Ammophila arenaria*	Poaceae	Grass	Perennial	Wetlands	Soilborne pathogen	Van der Putten et al., 1993, Troelstra et al., 2001, Knevel et al., 2004, Klironomos 2002, Beckstead and Parker 2003
31	*Ammophila breviligulata*	Poaceae	Grass	Perennial	Wetlands	Not reported	Danin 1997
32	*Ampelodesmos mauritanicus*	Poaceae	Grass	Perennial	Grassland	Autotoxicity	Mazzoleni et al., 2015

33	*Anacardium excelsum*	Anacardiaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Not reported	Kiers et al., 2000
34	*Anastatica hierochuntica*	Brassicaceae	Forb	Annual	Grassland	Autotoxicity	Hegazy et al., 1990
35	*Andropogon gerardii*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Holah and Alexander 1999, Gustafson and Casper 2004, Fitzsimons and Miller 2010, Casper et al., 2008, Casper and Castelli 2007, Bauer et al., 2015
36	*Antennaria microphylla*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Maron et al., 2016
37	*Anthericum ramosum*	Asparagaceae	Forb	Perennial	Grassland	Microbial shift	Hemrová et al., 2016
38	*Anthoxanthum odoratum*	Poaceae	Grass	Perennial	Grassland	Microbial shift/Soilborne pathogen/Nutrient imbalance or depletion	Zhang et al., 2016, Newman and Rovira 1975, Jing et al., 2015, Hendriks et al., 2013, Heinze et al., 2016, De Deyn et al., 2004, Cortois et al., 2016, Bezemer et al., 2006a, Bever 1994, Bergmann et al., 2016
39	*Apeiba membranacea*	Asteraceae	Forb	Perennial	Tropical/subtropical forest	Microbial shift	McCarthy-Neumann and Kobe 2010b
40	*Apera spica-venti*	Poaceae	Grass	Annual	Grassland	Microbial shift	Kardol et al., 2007
41	*Apocynum cannabinum*	Apocynaceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
42	*Arabidopsis thaliana*	Brassicaceae	Forb	Annual	Grassland	Microbial shift/Autotoxicity	Bukowski and Petermann 2014, Mazzoleni et al., 2015
43	*Arabis holboellii*	Brassicaceae	Forb	Perennial	Grassland	Microbial shift	Maron et al., 2016
44	*Araucaria cunninghamii*	Araucariaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Autotoxicity	Bevege 1968
45	*Arctium tomentosum*	Brassicaceae	Forb	Annual	Grassland	Soilborne pathogen	Petermann et al., 2008
46	*Ardisia quinquegona*	Primulaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Soilborne pathogen	Liang et al., 2016
47	*Aristida meridionalis*	Poaceae	Grass	Perennial	Semiarid/arid	Microbial shift	Van der Putten et al., 2007

48	*Arnica montana*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Jing et al., 2015
49	*Arrhenatherum elatius*	Poaceae	Grass	Annual	Grassland	Microbial shift/Soilborne pathogen	Zhang et al., 2016, Petermann et al., 2008, Heinze et al., 2016
50	*Artemisia biennis*	Asteraceae	Forb	Biennial	Grassland	Nutrient imbalance or depletion	Agrawal et al., 2005
51	*Artemisia californica*	Asteraceae	Forb	Biennial	Grassland	Microbial shift/Nutrient imbalance or depletion	Yelenik and Levine 2011, Valliere and Allen 2016, Sigüenza et al., 2006
52	*Artemisia campestris*	Asteraceae	Forb	Biennial	Grassland	Nutrient imbalance or depletion	Agrawal et al., 2005
53	*Artemisia capillaris*	Asteraceae	Forb	Biennial	Grassland	Microbial shift/Nutrient imbalance or depletion	Jiang et al., 2010
54	*Artemisia frigida*	Asteraceae	Forb	Biennial	Grassland	Microbial shift/Nutrient imbalance or depletion	Jiang et al., 2010
55	*Artemisia lavandulifolia*	Asteraceae	Forb	Biennial	Grassland	Microbial shift/Nutrient imbalance or depletion	Jiang et al., 2010
56	*Asarum canadense*	Aristolochiaceae	Forb	Perennial	Temperate forest	Not reported	Smith and Reynolds 2015
57	*Asclepias syriaca*	Asclepiadaceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
58	*Asclepias tuberosa*	Asclepiadaceae	Forb	Perennial	Grassland	Microbial shift	Fitzsimons and Miller 2010
59	*Asparagus officinalis*	Asparagaceae	Forb	Perennial	Grassland	Microbial shift/Autotoxicity	Young 1984, Klironomos 2002
60	*Aster novae-angliae*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
61	*Aster simplex*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
62	*Aster vimineus*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
63	*Avena barbata*	Poaceae	Grass	Annual	Grassland	Microbial shift	Yelenik and Levine 2011
64	*Avena fatua*	Poaceae	Grass	Annual	Grassland	Microbial shift/nutrient imbalance or depletion	Larios and Suding 2015
65	*Bellis perennis*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Cortois et al., 2016
66	*Berberis thunbergii*	Berberidaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Elgersma et al., 2012

67	*Berteroa incana*	Brassicaceae	Forb	Annual	Grassland	Soilborne pathogen	Petermann et al., 2008
68	*Betula alleghaniensis*	Betulaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
69	*Betula lenta*	Betulaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
70	*Betula papyrifera*	Betulaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
71	*Boswellia neglecta*	Burseraceae	Tree/Shrub	Perennial	Semiarid/arid	Microbial shift	Rutten et al., 2016
72	*Bothriochloa ischaemum*	Poaceae	Grass	Perennial	Grassland	Not reported	Kulmatiski et al., 2016
73	*Bothriochloa saccharoides*	Poaceae	Grass	Perennial	Grassland	Not reported	Kulmatiski et al., 2016
74	*Bouteloua curtipendula*	Poaceae	Grass	Perennial	Grassland	Not reported	Kulmatiski et al., 2016
75	*Bouteloua eriopoda*	Poaceae	Grass	Perennial	Semiarid/arid	Microbial shift	Chung and Rudgers 2016
76	*Bouteloua gracilis*	Poaceae	Grass	Perennial	Semiarid/arid	Microbial shift	Chung and Rudgers 2016
77	*Brachypodium pinnatum*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Hemrová et al., 2016
78	*Brachypodium rupestre*	Poaceae	Grass	Perennial	Grassland	Not reported	Bonanomi and Allegrezza 2004
79	*Briza media*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Jing et al., 2015, Bezemer et al., 2006a, Bergmann et al., 2016
80	*Bromus catharticus*	Poaceae	Grass	Perennial	Semiarid/arid	Microbial shift	Chiuffo et al., 2015
81	*Bromus erectus*	Poaceae	Grass	Perennial	Grassland	Microbial shift/ Nutrient imbalance or depletion	Hemrová et al., 2016, Ehlers and Thompson 2004, Cortois et al., 2016, Bezemer et al., 2006a, Bezemer et al., 2006b
82	*Bromus hordeaceus*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Cortois et al., 2016
83	*Bromus inermis*	Poaceae	Grass	Perennial	Grassland	Microbial shift/ Nutrient imbalance or depletion	Klironomos 2002, Jordan et al., 2008, Anacker et al., 2014, Agrawal et al., 2005

84	*Bromus kalmii*	Poaceae	Grass	Perennial	Grassland	Nutrient imbalance or depletion	Agrawal et al., 2005
85	*Bromus pubescens*	Poaceae	Grass	Perennial	Temperate forest	Not reported	Smith and Reynolds 2015
86	*Bromus sterilis*	Poaceae	Grass	Perennial	Grassland	Soilborne pathogen	Petermann et al., 2008
87	*Calluna vulgaris*	Ericaceae	Tree/Shrub	Perennial	Grassland	Autotoxicity	Bonanomi et al., 2005a
88	*Campanula patula*	Campanulaceae	Forb	Biennial	Grassland	Microbial shift	Cortois et al., 2016
89	*Campanula rapunculoides*	Campanulaceae	Forb	Biennial	Grassland	Nutrient imbalance or depletion	Agrawal et al., 2005
90	*Campanula rotundifolia*	Campanulaceae	Forb	Biennial	Grassland	Microbial shift/Nutrient imbalance or depletion	Jing et al., 2015, De Deyn et al., 2004, Agrawal et al., 2005
91	*Canarium album*	Burseraceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Soilborne pathogen	Liang et al., 2016
92	*Capsella bursa-pastoris*	Brassicaceae	Forb	Perennial	Grassland	Microbial shift	Schittko et al., 2016, Kardol et al., 2007, Jing et al., 2015
93	*Cardamine pensylvanica*	Brassicaceae	Forb	Biennial	Grassland	Autotoxicity	Molofsky et al., 2000
94	*Cardamine pratensis*	Brassicaceae	Forb	Biennial	Grassland	Microbial shift	Cortois et al., 2016
95	*Carduus nutans*	Asteraceae	Forb	Perennial	Semiarid/arid	Microbial shift	Chiuffo et al., 2015
96	*Carex arenaria*	Cyperaceae	Sedge	Perennial	Wetlands	Microbial shift/Soilborne pathogen	Van der Putten et al., 1993, Troelstra et al., 2001, Olff et al., 2000
97	*Carex aurea*	Cyperaceae	Sedge	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
98	*Carex flava*	Cyperaceae	Sedge	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
99	*Carex garberi*	Cyperaceae	Sedge	Perennial	Grassland	Microbial shift	Klironomos 2002
100	*Carex granularis*	Cyperaceae	Sedge	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
101	*Carex striata*	Cyperaceae	Sedge	Perennial	Wetlands	Autotoxicity	Koppel and Crain 2006
102	*Carpinus caroliniana*	Betulaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Keymer and Lankau 2016
103	*Carum carvi*	Cyperaceae	Sedge	Perennial	Grassland	Microbial shift	Cortois et al., 2016

104	*Carya cordiformis*	Juglandaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
105	*Carya glabra*	Juglandaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
106	*Carya ovata*	Juglandaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
107	*Carya tomentosa*	Juglandaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
108	*Castanopsis fabri*	Fagaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Soilborne pathogen	Liang et al., 2016
109	*Castanopsis fissa*	Fagaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Soilborne pathogen	Liang et al., 2016
110	*Cenchrus biflorus*	Poaceae	Grass	Annual	Semiarid/arid	Microbial shift	Van der Putten et al., 2007
111	*Cenchrus spinifex*	Poaceae	Grass	Annual	Semiarid/arid	Microbial shift	Chiuffo et al., 2015
112	*Centaurea jacea*	Asteraceae	Forb	Perennial	Grassland	Microbial shift/Soilborne pathogen/Nutrient imbalance or depletion	Petermann et al., 2008, Klironomos 2002, Jing et al., 2015, Frouz et al., 2016, De Deyn et al., 2004, Cortois et al., 2016, Bergmann et al., 2016, Anacker et al., 2014
113	*Centaurea maculosa*	Asteraceae	Forb	Perennial	Grassland	Microbial shift/Autotoxicity	Meiman et al., 2006, Perry et al., 2005, Callaway et al., 2004
114	*Centaurea solstitialis*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	King 2015, Chiuffo et al., 2015
115	*Centaurea stoebe*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Maron et al., 2014
116	*Cerastium arvense*	Caryophyllaceae	Forb	Perennial	Grassland	Nutrient imbalance or depletion	Agrawal et al., 2005
117	*Cerastium fontanum*	Caryophyllaceae	Forb	Perennial	Grassland	Nutrient imbalance or depletion	Agrawal et al., 2005
118	*Cerastium vulgatum*	Caryophyllaceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
119	*Chamaecrista fasciculata*	Fabaceae	Forb	Annual	Grassland	Microbial shift	Holah and Alexander 1999
120	*Chenopodium album*	Chenopodiaceae	Forb	Biennial	Semiarid/arid	Microbial shift	Chiuffo et al., 2015
121	*Chenopodium ambrosioides*	Chenopodiaceae	Forb	Biennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014

122	*Chromolaena odorata*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Te Beest et al., 2009
123	*Chrysanthemum leucanthemum*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
124	*Cichorium intybus*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Schittko et al., 2016, Klironomos 2002, Anacker et al., 2014
125	*Cinnamomum verum*	Lauracee	Tree/Shrub	Perennial	Tropical/subtropical forest	Nutrient imbalance or depletion	Kueffer et al., 2009
126	*Cirsium arvense*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Rice 1984, Klironomos 2002, Anacker et al., 2014
127	*Cirsium oleraceum*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Cortois et al., 2016
128	*Cirsium palustre*	Asteraceae	Forb	Perennial	Wetlands	Autotoxicity	Ballegaard and Warncke 1985
129	*Cirsium vulgare*	Asteraceae	Forb	Perennial	Grassland	Microbial shift/ Autotoxicity/ Nutrient imbalance or depletion	Klironomos 2002, De Jong and Klinkhamer 1985, Anacker et al., 2014
130	*Colubrina spinosa*	Solanaceae	Forb	Annual	Tropical/subtropical forest	Microbial shift	McCarthy-Neumann and Kobe 2010b
131	*Combretum molle*	Combretaceae	Tree/Shrub	Perennial	Semiarid/arid	Microbial shift	Rutten et al., 2016
132	*Conoclinium coelestinum*	Asteraceae	Forb	Perennial	Temperate forest	Not reported	Smith and Reynolds 2015
133	*Convolvulus arvensis*	Convolvulaceae	Forb	Annual	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
134	*Conyza bonariensis*	Asteraceae	Forb	Annual	Grassland	Microbial shift	Kardol et al., 2007, Chiuffo et al., 2015
135	*Cornus controversa*	Cornaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bayandala et al., 2016
136	*Cornus florida*	Cornaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
137	*Coronilla varia*	Fabaceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
138	*Crepis biennis*	Asteraceae	Forb	Biennial	Grassland	Microbial shift	Zuppinger-Dingley et al., 2016, Cortois et al., 2016
139	*Crepis capillaris*	Asteraceae	Forb	Annual	Grassland	Microbial shift	Jing et al., 2015

140	*Cryptocarya concinna*	Lauracee	Tree/Shrub	Perennial	Tropical/subtropical forest	Soilborne pathogen	Liang et al., 2016
141	*Cunninghamia lanceolata*	Cupressaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Autotoxicity	Zhang 1993, Xia et al., 2016, Chen et al., 2005
142	*Cupressus sempervirens*	Cupressaceae	Tree/Shrub	Perennial	Temperate forest	Autotoxicity	Mazzoleni et al., 2015
143	*Cynosurus cristatus*	Poaceae	Grass	Perennial	Grassland	Autotoxicity	Newman and Rovira 1975, Bever 1994
144	*Dactylis glomerata*	Poaceae	Grass	Perennial	Grassland	Microbial shift/Soilborne pathogen/Autotoxicity/Nutrient imbalance or depletion	Petermann et al., 2008, Klironomos 2002, Grant and Sallans 1964, Cortois et al., 2016, Bergmann et al., 2016, Anacker et al., 2014
145	*Danthonia spicata*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Bever 1994
146	*Danthonia unispicata*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Maron et al., 2016
147	*Daucus carota*	Apiaceae	Forb	Biennial	Grassland	Microbial shift	Klironomos 2002, Frouz et al., 2016, Cortois et al., 2016, Bergmann et al., 2016, Anacker et al., 2014
148	*Daucus pusillus*	Apiaceae	Forb	Biennial	Semiarid/arid	Microbial shift	Chiuffo et al., 2015
149	*Deschampsia caespitosa*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Suding et al., 2004
150	*Deschampsia flexuosa*	Poaceae	Grass	Perennial	Grassland	Soilborne pathogen	Zhang et al., 2016
151	*Digitaria sanguinalis*	Poaceae	Grass	Annual	Grassland	Autotoxicity	Parenti and Rice 1969
152	*Dipteryx panamensis*	Fabaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Not reported	Kiers et al., 2000
153	*Dittrichia viscosa*	Asteraceae	Tree/Shrub	Perennial	Grassland	Nutrient imbalance or depletion	Bonanomi and Mazzoleni 2005
154	*Echinacea purpurea*	Asteraceae	Forb	Perennial	Grassland	Not reported	Kulmatiski et al., 2016
155	*Echinochloa crus-galli*	Poaceae	Grass	Annual	Grassland	Microbial shift/Soilborne pathogen	Petermann et al., 2008, Jing et al., 2015
156	*Echium vulgare*	Boraginaceae	Forb	Biennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014

157	*Elaeagnus umbellata*	Elaeagnaceae	Tree/Shrub	Perennial	Shrubland	Microbial shift	Shannon et al., 2014
158	*Elymus athercus*	Poaceae	Grass	Perennial	Wetlands	Soilborne pathogen	Van der Putten et al., 1993
159	*Elymus canadensis*	Poaceae	Grass	Perennial	Grassland	Soilborne pathogen	Bauer et al., 2015
160	*Elymus elymoides*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Perkins and Nowak 2012
161	*Elymus hystrix*	Poaceae	Grass	Perennial	Temperate forest	Not reported	Smith and Reynolds 2015
162	*Elymus repens*	Poaceae	Grass	Perennial	Grassland	Nutrient imbalance or depletion	Agrawal et al., 2005
163	*Elymus trachycaulus*	Poaceae	Grass	Perennial	Grassland	Nutrient imbalance or depletion	Agrawal et al., 2005
164	*Engelhardtia fenzelii*	Juglandaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Soilborne pathogen	Liang et al., 2016
165	*Eragrostis lehmanniana*	Poaceae	Grass	Perennial	Semiarid/arid	Microbial shift	Van der Putten et al., 2007
166	*Erigeron canadensis*	Asteraceae	Forb	Annual	Grassland	Not reported	Keever 1950
167	*Erigeron philadelphicus*	Asteraceae	Forb	Annual	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
168	*Erigeron strigosus*	Asteraceae	Forb	Annual	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
169	*Eriogonum arborescens*	Polygonaceae	Tree/Shrub	Perennial	Grassland	Microbial shift	Yelenik and Levine 2011, Xia et al., 2016
170	*Erodium macrophyllum*	Geraniaceae	Forb	Annual	Grassland	Microbial shift	Gillespie and Allen 2006
171	*Eucalyptus globulus*	Myrtaceae	Tree/Shrub	Perennial	Semiarid/arid	Autotoxicity	Moral and Cates 1971
172	*Eucalyptus pilularis*	Myrtaceae	Tree/Shrub	Perennial	Semiarid/arid	Not reported	Florence and Crocker 1962
173	*Euonymus fortunei*	Celastraceae	Tree/Shrub	Perennial	Temperate forest	Not reported	Smith and Reynolds 2015
174	*Eupatorium fortunei*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Niu et al., 2007
175	*Euphorbia esula*	Euphorbiaceae	Forb	Perennial	Grassland	Microbial shift	Maron et al., 2014, Jordan et al., 2008

176	*Fagus grandifolia*	Fagaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
177	*Fagus sylvatica*	Fagaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Wurst et al., 2015
178	*Festuca drymeia*	Poaceae	Grass	Perennial	Grassland	Autotoxicity	Mazzoleni et al., 2015
179	*Festuca filiformis*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Jing et al., 2015
180	*Festuca idahoensis*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Maron et al., 2016
181	*Festuca ovina*	Poaceae	Grass	Perennial	Grassland	Microbial shift	De Deyn et al., 2004, Bezemer et al., 2006a
182	*Festuca pratensis*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Zuppinger-Dingley et al., 2016, Cortois et al., 2016
183	*Festuca rubra*	Poaceae	Grass	Perennial	Grassland	Microbial shift/Soilborne Pathogen/Nutrient imbalance or depletion	Wubs and Bezemer 2016, Petermann et al., 2008, Olff et al., 2000, Hendriks et al., 2013, Frouz et al., 2016, De Deyn et al., 2004, Cortois et al., 2016
184	*Fragaria virginiana*	Rosaceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
185	*Fraxinus americana*	Oleaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	McCarthy-Neumann and Kobe 2010a, Bennett et al., 2017
186	*Gaillardia megapotamica*	Asteraceae	Forb	Perennial	Semiarid/arid	Microbial shift	Chiuffo et al., 2015
187	*Galium mollugo*	Rubiaceae	Forb	Perennial	Grassland	Microbial shift/Soilborne pathogen	Zuppinger-Dingley et al., 2016, Petermann et al., 2008, Klironomos 2002, Cortois et al., 2016, Anacker et al., 2014
188	*Galium palustre*	Rubiaceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
189	*Gentiana alba*	Gentianaceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002
190	*Geranium pratense*	Geraniaceae	Forb	Perennial	Grassland	Microbial shift	Cortois et al., 2016
191	*Geum aleppicum*	Rosaceae	Forb	Perennial	Grassland	Microbial shift/Nutrient imbalance or depletion	Klironomos 2002, Anacker et al., 2014, Agrawal et al., 2005
192	*Geum canadense*	Rosaceae	Forb	Perennial	Grassland	Microbial shift	Fitzpatrick et al., 2016

193	*Geum triflorum*	Rosaceae	Forb	Perennial	Grassland	Microbial shift	Maron et al., 2016
194	*Geum urbanum*	Rosaceae	Forb	Perennial	Grassland	Nutrient imbalance or depletion	Agrawal et al., 2005
195	*Grevillea robusta*	Proteaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Autotoxicity	Webb et al., 1967
196	*Hedeoma hispida*	Lamiaceae	Forb	Perennial	Grassland	Microbial shift	Fitzpatrick et al., 2016
197	*Hedera helix*	Araliaceae	Vine	Perennial	Temperate forest	Autotoxicity	Mazzoleni et al., 2015
198	*Helianthus annuus*	Asteraceae	Forb	Annual	Grassland	Microbial shift/Autotoxicity	Rice 1984, Lou et al., 2014
199	*Helianthus occidentalis*	Asteraceae	Forb	Perennial	Grassland	Autotoxicity	Curtis and Cottam 1950
200	*Helianthus rigidus*	Asteraceae	Forb	Perennial	Grassland	Autotoxicity	Curtis and Cottam 1950
201	*Heracleum mantegazzianum*	Apiaceae	Forb	Annual	Grassland	Microbial shift	Van Grunsven et al., 2007
202	*Heracleum sphondylium*	Apiaceae	Forb	Perennial	Grassland	Microbial shift	Van Grunsven et al., 2007
203	*Hieracium auranticum*	Apiaceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
204	*Hieracium pilosella*	Apiaceae	Forb	Perennial	Grassland	Microbial shift	Lamoureaux et al. 2003, Klironomos 2002, Anacker et al., 2014
205	*Hieracium pratense*	Apiaceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002
206	*Hilaria jamesii*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Belnap et al., 2005
207	*Hippophaë rhamnoides*	Elaeagnaceae	Tree/Shrub	Perennial	Semiarid/arid	Soilborne pathogen	Oremus and Otten 1983
208	*Hirschfeldia incana*	Brassicaceae	Forb	Annual	Semiarid/arid	Microbial shift	Chiuffo et al., 2015
209	*Holcus lanatus*	Poaceae	Grass	Perennial	Grassland	Microbial shift/Soilborne pathogen/Autotoxicity	Zhang et al., 2016, Petermann et al., 2008, Newman and Rovira 1975, Jing et al., 2015, Heinze et al., 2016, Cortois et al., 2016, Bonanomi and Mazzoleni 2005, Bergmann et al., 2016
210	*Holcus mollis*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Jing et al., 2015

211	*Hordeum murinum*	Poaceae	Grass	Perennial	Grassland	Soilborne pathogen	Petermann et al., 2008
212	*Hordeum stenostachys*	Poaceae	Grass	Perennial	Semiarid/arid	Microbial shift	Chiuffo et al., 2015
213	*Hypericum perforatum*	Clusiaceae	Forb	Perennial	Grassland	Microbial shift	Maron et al., 2014, Klironomos 2002, Jing et al., 2015, Anacker et al., 2014
214	*Hypochaeris radicata*	Asteraceae	Forb	Perennial	Grassland	Microbial shift/Soilborne pathogen/Autotoxicity/Nutrient imbalance or depletion	Zhang et al., 2016, Wubs and Bezemer 2016, Newman and Rovira 1975, Jing et al., 2015, Chiuffo et al., 2015, Bezemer et al., 2006a, Bever 1994
215	*Inula salicina*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Hemrová et al., 2016
216	*Iriartea deltoidea*	Arecaceae	Forb	Annual	Tropical/subtropical forest	Microbial shift	McCarthy-Neumann and Kobe 2010b
217	*Jacobaea vulgaris*	Asteraceae	Forb	Perennial	Grassland	Microbial shift/Soilborne pathogen/Nutrient imbalance or depletion	Zhang et al., 2016, Wubs and Bezemer 2016, Kos et al., 2015b, Kos et al., 2015a
218	*Juncus dudlei*	Juncaceae	Sedge	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
219	*Juncus effusus*	Juncaceae	Sedge	Perennial	Wetlands	Autotoxicity	Ervin and Wetzel 2000
220	*Juniperus virginiana*	Cupressaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
221	*Kalanchoe daigremontiana*	Crassulaceae	Forb	Perennial	Tropical/subtropical forest	Autotoxicity	Groner 1974
222	*Knautia arvensis*	Dipsacaceae	Forb	Perennial	Grassland	Microbial shift	Cortois et al., 2016
223	*Kochia scoparia*	Amaranthaceae	Forb	Annual	Semiarid/arid	Not reported	Rice 1984, Lodhi 1979b
224	*Koeleria macrantha*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Maron et al., 2016
225	*Krigia dandelion*	Asteraceae	Forb	Annual	Grassland	Microbial shift	Bever 1994
226	*Kummerovia stipulacea*	Fabaceae	Forb	Annual	Grassland	Microbial shift/Soilborne pathogen	Van der Putten et al., 2001
227	*Lantana camara*	Verbenaceae	Forb	Annual	Grassland	Autotoxicity	Arora et al. 1993
228	*Larix lacricina*	Pinaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017

229	*Lasthenia californica*	Asteraceae	Forb	Annual	Grassland	Nutrient imbalance or depletion	Petermann et al., 2008
230	*Lathyrus pratensis*	Fabaceae	Forb	Perennial	Grassland	Microbial shift	Cortois et al., 2016
231	*Leontodon autumnalis*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Cortois et al., 2016
232	*Leontodon hispidus*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Cortois et al., 2016
233	*Lepidium bonariense*	Brassicaceae	Forb	Annual	Semiarid/arid	Microbial shift	Chiuffo et al., 2015
234	*Lepidium campestre*	Brassicaceae	Forb	Annual	Grassland	Soilborne pathogen/Nutrient imbalance or depletion	Petermann et al., 2008, Agrawal et al., 2005
235	*Lepidium densiflorum*	Brassicaceae	Forb	Annual	Grassland	Microbial shift/Nutrient imbalance or depletion	Fitzpatrick et al., 2016, Agrawal et al., 2005
236	*Lepidium sativum*	Brassicaceae	Forb	Annual	Wetlands	Autotoxicity	Mazzoleni et al., 2015
237	*Lespedeza cuneata*	Fabaceae	Forb	Perennial	Grassland	Microbial shift	Crawford and Knight 2016
238	*Leucanthemum vulgare*	Asteraceae	Forb	Perennial	Grassland	Microbial shift/Soilborne pathogen/Nutrient imbalance or depletion	Zhang et al., 2016, Petermann et al., 2008, Maron et al., 2014, Jing et al., 2015, Hendriks et al., 2013, Cortois et al., 2016, Bergmann et al., 2016
239	*Leymus chinensis*	Poaceae	Grass	Perennial	Grassland	Microbial shift/Nutrient imbalance or depletion	Jiang et al., 2010
240	*Liatris spicata*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002
241	*Licania platypus*	Chrysobalanaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Not reported	Kiers et al., 2000
242	*Ligustrum sinense*	Oleaceae	Tree/Shrub	Perennial	Temperate forest	Not reported	Kuebbing et al., 2015
243	*Ligustrum vulgare*	Oleaceae	Tree/Shrub	Perennial	Shrubland	Microbial shift	Shannon et al., 2014
244	*Linaria vulgaris*	Scrophulariaceae	Forb	Perennial	Grassland	Microbial shift	Maron et al., 2014, Klironomos 2002, Anacker et al., 2014
245	*Lindera benzoin*	Lauracee	Tree/Shrub	Perennial	Temperate forest	Not reported	Smith and Reynolds 2015
246	*Liriodendron tulipifera*	Magnoliaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Nagendra and Peterson 2016, Bennett et al., 2017

247	*Litsea elongata*	Lauracee	Tree/Shrub	Perennial	Tropical/subtropical forest	Soilborne pathogen	Liang et al., 2016
248	*Lolium multiflorum*	Poaceae	Grass	Perennial	Grassland	Microbial shift	García-Parisi and Omacini 2017
249	*Lolium perenne*	Poaceae	Grass	Perennial	Grassland	Microbial shift/Soilborne pathogen/Autotoxicity	Zhang et al., 2016, Niu et al., 2007, Newman and Rovira 1975, Klironomos 2002, Jing et al., 2015, De Deyn et al., 2004
250	*Lolium rigidum*	Poaceae	Grass	Perennial	Grassland	Autotoxicity	Canals et al., 2005
251	*Lonicera maackii*	Caprifoliaceae	Tree/Shrub	Perennial	Shrubland	Microbial shift	Shannon et al., 2014, Kuebbing et al., 2015
252	*Lotus corniculatus*	Fabaceae	Forb	Perennial	Grassland	Microbial shift/Autotoxicity/ Nutrient imbalance or depletion	Wubs and Bezemer 2016, Grant and Sallans 1964, Frouz et al., 2016, Cortois et al., 2016
253	*Luchea seemannii*	Fabaceae	Forb	Perennial	Tropical/subtropical forest	Not reported	Kiers et al., 2000
254	*Lupinus sericeus*	Fabaceae	Forb	Perennial	Grassland	Microbial shift	Maron et al., 2016
255	*Medicago lupulina*	Fabaceae	Forb	Perennial	Grassland	Microbial shift/Soilborne pathogen	Petermann et al., 2008, Klironomos 2002, Cortois et al., 2016, Anacker et al., 2014
256	*Medicago marina*	Fabaceae	Forb	Perennial	Grassland	Autotoxicity	Bonanomi et al., 2007
257	*Medicago sativa*	Fabaceae	Forb	Perennial	Grassland	Microbial shift/Autotoxicity	Niu et al., 2007, Miller 1996, Mazzoleni et al., 2015
258	*Medicago varia*	Fabaceae	Forb	Perennial	Grassland	Microbial shift	Schittko et al., 2016, Cortois et al., 2016
259	*Megathyrsus maximus*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Te Beest et al., 2009
260	*Melilotus albus*	Fabaceae	Forb	Perennial	Grassland	Microbial shift/Soilborne pathogen	Schittko and Wurst 2014, Petermann et al., 2008
261	*Milicia regia*	Moraceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Soilborne pathogen	Hood et al., 2004
262	*Nardus stricta*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Jing et al., 2015
263	*Nassella leucotricha*	Poaceae	Grass	Perennial	Grassland	Not reported	Kulmatiski et al., 2016

264	*Nyssa sylvatica*	Cornaceae	Tree/Shrub	Perennial	Temperate forest	Not reported	Nagendra and Peterson 2016
265	*Ochroma pyramidale*	Bombacaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Not reported	Kiers et al., 2000
266	*Oenothera biennis*	Onagraceae	Forb	Annual	Grassland	Microbial shift	Fitzpatrick et al., 2016, Anacker et al., 2014
267	*Oenothera perennis*	Onagraceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
268	*Oenothera speciosa*	Onagraceae	Forb	Perennial	Grassland	Not reported	Kulmatiski et al., 2016
269	*Onobrychis vincifolia*	Fabaceae	Forb	Perennial	Grassland	Microbial shift	Zuppinger-Dingley et al., 2016, Cortois et al., 2016
270	*Ormosia glaberrima*	Fabaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Soilborne pathogen	Liang et al., 2016
271	*Ostrya virginiana*	Betulaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
272	*Oxydendrum arboreum*	Ericaceae	Tree/Shrub	Perennial	Temperate forest	Not reported	Nagendra and Peterson 2016
273	*Ozoroa insignis*	Anacardaceae	Tree/Shrub	Perennial	Semiarid/arid	Microbial shift	Rutten et al., 2016
274	*Panicum capillare*	Poaceae	Grass	Annual	Grassland	Soilborne pathogen	Petermann et al., 2008
275	*Panicum coloratum*	Poaceae	Grass	Annual	Grassland	Not reported	Kulmatiski et al., 2016
276	*Panicum lanuginosum*	Poaceae	Grass	Annual	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
277	*Panicum sphaerocarpum*	Poaceae	Grass	Annual	Grassland	Microbial shift	Bever 1994
278	*Panicum urvilleanum*	Poaceae	Grass	Annual	Semiarid/arid	Not reported	Danin 1997
279	*Panicum virgatum*	Poaceae	Grass	Annual	Grassland	Soilborne pathogen	Bauer et al., 2015
280	*Parthenium hysterophorus*	Asteraceae	Forb	Perennial	Grassland	Autotoxicity	Picman and Picman 1984
281	*Parthenium integrifolium*	Asteraceae	Forb	Perennial	Grassland	Soilborne pathogen	Bauer et al., 2015
282	*Pastinaca sativa*	Apiaceae	Forb	Biennial	Grassland	Microbial shift	Cortois et al., 2016

283	*Pennisetum centrasiaticum*	Poaceae	Grass	Perennial	Grassland	Microbial shift/Nutrient imbalance or depletion	Jiang et al., 2010
284	*Pentaclethra macroloba*	Fabaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Microbial shift	McCarthy-Neumann and Kobe 2010b
285	*Petrorhagia velutina*	Caryophyllaceae	Forb	Perennial	Grassland	Not reported	Mazzoleni et al., 2007
286	*Phleum pratense*	Poaceae	Grass	Perennial	Grassland	Microbial shift/Autotoxicity	Klironomos 2002, Fitzpatrick et al., 2016, Cortois et al., 2016, Anacker et al., 2014
287	*Phragmites australis*	Poaceae	Grass	Perennial	Wetlands	Autotoxicity	Gopal and Goel 1993, Armstrong and Armstrong 2001
288	*Phytolacca americana*	Phytolaccaceae	Forb	Biennial	Temperate forest	Autotoxicity	Edwards and Fletcher 1988
289	*Picea abies*	Pinaceae	Tree/Shrub	Perennial	Boreal forest	Autotoxicity	Pellissier and Souto 1999
290	*Picea glauca*	Pinaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
291	*Picea mariana*	Pinaceae	Tree/Shrub	Perennial	Boreal forest	Microbial shift/Autotoxicity	Mallik and Newton 1988, Bennett et al., 2017
292	*Picea rubens*	Pinaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
293	*Picea sitchensis*	Pinaceae	Tree/Shrub	Perennial	Boreal forest	Not reported	Chapin et al., 1994
294	*Pimpinella major*	Apiaceae	Forb	Perennial	Grassland	Microbial shift	Cortois et al., 2016
295	*Pinus contorta*	Pinaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Gundale et al., 2014
296	*Pinus halepensis*	Pinaceae	Tree/Shrub	Perennial	Temperate forest	Autotoxicity	Mazzoleni et al., 2015
297	*Pinus monticola*	Pinaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
298	*Pinus radiata*	Pinaceae	Tree/Shrub	Perennial	Semiarid/arid	Not reported	Rice 1984, Chu-Chou 1978
299	*Pinus resinosa*	Pinaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
300	*Pinus strobus*	Pinaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Nagendra and Peterson 2016, Bennett et al., 2017
301	*Pinus taeda*	Pinaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Nijjer et al., 2007, Bennett et al., 2017

302	*Pinus virginiana*	Pinaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
303	*Plantago erecta*	Plantaginaceae	Forb	Perennial	Grassland	Nutrient imbalance or depletion	Batten et al., 2008
304	*Plantago lanceolata*	Plantaginaceae	Forb	Perennial	Grassland	Microbial shift/Soilborne Pathogen/Autotoxicity/Nutrient imbalance or depletion	Zuppinger-Dingley et al., 2016, Zhang et al., 2016, Stanescu and Maherali 2016, Petermann et al., 2008, Newman and Rovira 1975, Klironomos 2002, Hendriks et al., 2013, Frouz et al., 2016, De Deyn et al., 2004, Cortois et al., 2016, Bezemer et al., 2006a, Bergmann et al., 2016, Anacker et al., 2014
305	*Plantago major*	Plantaginaceae	Forb	Perennial	Grassland	Microbial shift/Nutrient imbalance or depletion	Bergmann et al., 2016, Agrawal et al., 2005
306	*Plantago media*	Plantaginaceae	Forb	Perennial	Grassland	Microbial shift	Cortois et al., 2016
307	*Plantago rugelii*	Plantaginaceae	Forb	Perennial	Grassland	Microbial shift/Nutrient imbalance or depletion	Fitzpatrick et al., 2016, Agrawal et al., 2005
308	*Platanus occidentalis*	Pinaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
309	*Poa annua*	Poaceae	Grass	Annual	Grassland	Microbial shift	Van Grunsven et al., 2007, Kardol et al., 2007, Jing et al., 2015, Frouz et al., 2016
310	*Poa compressa*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
311	*Poa pratensis*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Zuppinger-Dingley et al., 2016, Klironomos 2002, Cortois et al., 2016, Anacker et al., 2014
312	*Poa secunda*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Maron et al., 2016
313	*Poa trivialis*	Poaceae	Grass	Perennial	Grassland	Microbial shift	De Deyn et al., 2004, Cortois et al., 2016
314	*Polygala incarnata*	Polygalaceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002
315	*Populus grandidentata*	Betulaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017

316	*Populus trihocarpa*	Salicaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Reinhart et al., 2005
317	*Potamogeton pectinatus*	Potamogetonaceae	Forb	Perennial	Grassland	Nutrient imbalance or depletion	Bodelier et al., 2006
318	*Potentilla arguta*	Rosaceae	Forb	Perennial	Grassland	Nutrient imbalance or depletion	Agrawal et al., 2005
319	*Potentilla recta*	Rosaceae	Forb	Perennial	Grassland	Microbial shift/Nutrient imbalance or depletion	Maron et al., 2014, Klironomos 2002, Anacker et al., 2014, Agrawal et al., 2005
320	*Prestoea decurrens*	Ranunculaceae	Forb	Annual	Tropical/subtropical forest	Microbial shift	McCarthy-Neumann and Kobe 2010b
321	*Prosopis juliflora*	Fabaceae	Tree/Shrub	Perennial	Semiarid/arid	Autotoxicity	Warrag 1995
322	*Prunella vulgaris*	Lamiaceae	Forb	Perennial	Grassland	Microbial shift	Zuppinger-Dingley et al., 2016, Klironomos 2002, De Deyn et al., 2004, Cortois et al., 2016, Bezemer et al., 2006a, Anacker et al., 2014
323	*Prunus grayana*	Rosaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bayandala et al., 2016
324	*Prunus pensylvanica*	Rosaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
325	*Prunus serotina*	Rosaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift/Soilborne pathogen	Reinhart et al., 2003, Packer and Clay 2000
326	*Pseudoroegneria spicata*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Maron et al., 2016
327	*Pseudotsuga menziesii*	Pinaceae	Tree/Shrub	Perennial	Temperate forest	Soilborne pathogen	Jenny et al. 1997
328	*Pulicaria dysenterica*	Asteraceae	Forb	Perennial	Grassland	Not reported	Bonanomi and Mazzoleni 2005
329	*Quercus alba*	Fagaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Nagendra and Peterson 2016, Bennett et al., 2017
330	*Quercus ellipsoidalis*	Fagaceae	Tree/Shrub	Perennial	Temperate forest	Nutrient imbalance or depletion	Dickie et al., 2007
331	*Quercus falcata*	Fagaceae	Tree/Shrub	Perennial	Semiarid/arid	Not reported	Harborne 1972
332	*Quercus ilex*	Fagaceae	Tree/Shrub	Perennial	Temperate forest	Autotoxicity	Mazzoleni et al., 2015

333	*Quercus macrocarpa*	Fagaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
334	*Quercus nigra*	Fagaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Nijjer et al., 2007
335	*Quercus palustris*	Fagaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
336	*Quercus petraea*	Fagaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Wurst et al., 2015
337	*Quercus pubescens*	Fagaceae	Tree/Shrub	Perennial	Temperate forest	Autotoxicity	Mazzoleni et al., 2015
338	*Quercus rubra*	Fagaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	McCarthy-Neumann and Kobe 2010a
339	*Quercus velutina*	Fagaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
340	*Ranunculus acris*	Ranunculaceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Cortois et al., 2016, Anacker et al., 2014
341	*Ratibida columnifera*	Asteraceae	Forb	Perennial	Grassland	Not reported	Kulmatiski et al., 2016
342	*Ratibida pinnata*	Asteraceae	Forb	Perennial	Grassland	Microbial shift/Soilborne pathogen	Fitzsimons and Miller 2010, Bauer et al., 2015
343	*Robinia pseudoacacia*	Fagaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift/Autotoxicity	Bennett et al., 2017, Mazzoleni et al., 2015
344	*Rorippa austriaca*	Brassicaceae	Forb	Annual	Grassland	Microbial shift	Dostálek et al., 2016.
345	*Rudbeckia hirta*	Asteraceae	Forb	Annual	Grassland	Microbial shift/Soilborne pathogen	Fitzsimons and Miller 2010, Bauer et al., 2015
346	*Rudbeckia serotina*	Asteraceae	Forb	Biennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
347	*Rumex acetosa*	Polygonaceae	Forb	Perennial	Grassland	Microbial shift/Autotoxicity	Newman and Rovira 1975, Jing et al., 2015, Cortois et al., 2016
348	*Rumex acetosella*	Polygonaceae	Forb	Perennial	Grassland	Soilborne pathogen	Zhang et al., 2016
349	*Rumex crispus*	Polygonaceae	Forb	Perennial	Grassland	Microbial shift	Fitzpatrick et al., 2016, Chiuffo et al., 2015, Burns et al., 2017
350	*Rumex obtusifolius*	Polygonaceae	Forb	Perennial	Grassland	Microbial shift/Soilborne pathogen	Zhang et al., 2016, Jing et al., 2015, De Deyn et al., 2004,
351	*Salsola kali*	Chenopodiaceae	Tree/Shrub	Annual	Semiarid/arid	Microbial shift	Lodhi 1979a, Chiuffo et al., 2015
352	*Salvia azurea*	Lamiaceae	Forb	Perennial	Grassland	Not reported	Kulmatiski et al., 2016

353	*Sanguisorba minor*	Rosaceae	Forb	Perennial	Grassland	Microbial shift	Bezemer et al., 2006a
354	*Sanguisorba officinalis*	Rosaceae	Forb	Perennial	Grassland	Microbial shift	Cortois et al., 2016
355	*Sapium sebiferum*	Euphorbiaceae	Tree/Shrub	Perennial	Grassland	Microbial shift	Nijjer et al., 2007
356	*Satureja vulgaris*	Lamiaceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
357	*Schima superba*	Theaceae	Tree/Shrub	Perennial	Tropical/subtropical forest	Soilborne pathogen	Liang et al., 2016
358	*Schizachyrium scoparium*	Poaceae	Grass	Perennial	Wetlands	Microbial shift/Autotoxicity	Wedin and Tilman 1993, Tilman and Wedin 1991, Kulmatiski et al., 2016, Gustafson and Casper 2004, Casper et al., 2008, Casper and Castelli 2007
359	*Scirpus holoschoenus*	Cyperaceae	Sedge	Perennial	Grassland	Not reported	Bonanomi et al., 2005b
360	*Senecio jacobaea*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Bezemer et al., 2006a, Bezemer et al., 2006b
361	*Sequoia sempervirens*	Cupressaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Florence 1965
362	*Silene antirrhina*	Caryophyllaceae	Forb	Annual	Grassland	Nutrient imbalance or depletion	Agrawal et al., 2005
363	*Silene cucubalus*	Caryophyllaceae	Forb	Annual	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
364	*Silene vulgaris*	Caryophyllaceae	Forb	Annual	Grassland	Nutrient imbalance or depletion	Agrawal et al., 2005
365	*Sisymbrium loeselii*	Brassicaceae	Forb	Annual	Grassland	Not reported	Schittko et al., 2016
366	*Solanum carolinense*	Solanaceae	Forb	Annual	Grassland	Autotoxicity	Solomon 1983
367	*Solidago canadensis*	Asteraceae	Forb	Perennial	Grassland	Microbial shift/Nutrient imbalance or depletion	Schittko and Wurst 2014, Schittko et al., 2016, Sanderson et al., 2015, Pendergast et al., 2013, Klironomos 2002, Anacker et al., 2014
368	*Solidago graminifolia*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002
369	*Solidago nemoralis*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014

370	*Solidago rugosa*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Pendergast et al., 2013, Klironomos 2002, Anacker et al., 2014
371	*Sorghastrum nutans*	Poaceae	Grass	Perennial	Grassland	Microbial shift/Autotoxicity	Kulmatiski et al., 2016, Fitzsimons and Miller 2010, Castelli and Casper 2003, Casper et al., 2008, Casper and Castelli 2007, Gustafson and Casper 2004
372	*Sorghum halepense*	Poaceae	Grass	Perennial	Grassland	Autotoxicity	Abdul-Wahab and Rice 1967
373	*Sporobolus asper*	Poaceae	Grass	Perennial	Grassland	Not reported	Kulmatiski et al., 2016
374	*Sporobolus heterolepis*	Poaceae	Grass	Perennial	Grassland	Microbial shift/Soilborne pathogen	Casper et al., 2008, Bauer et al., 2015
375	*Sporobolus neglectus*	Poaceae	Grass	Perennial	Grassland	Microbial shift	Fitzpatrick et al., 2016
376	*Sporobolus pyramidalis*	Poaceae	Grass	Perennial	Semiarid/arid	Not reported	Rice 1984
377	*Stellaria media*	Caryophyllaceae	Forb	Biennial	Grassland	Microbial shift	De Deyn et al., 2004
378	*Stipa krylovii*	Poaceae	Grass	Perennial	Grassland	Nutrient imbalance or depletion	Jiang et al., 2010
379	*Stipa pulchra*	Poaceae	Grass	Perennial	Grassland	Microbial shift/nutrient imbalance or depletion	Larios and Suding 2015
380	*Stipagrostis spoparia*	Poaceae	Grass	Perennial	Semiarid/arid	Not reported	Danin 1997
381	*Succisa pratensis*	Dipsacaceae	Forb	Perennial	Grassland	Microbial shift	De Deyn et al., 2004
382	*Swallenia alexandrae*	Poaceae	Grass	Perennial	Semiarid/arid	Not reported	Danin 1997
383	*Symphyotrichum novae-angliae*	Asteraceae	Forb	Perennial	Grassland	Microbial shift/Soilborne pathogen	Pendergast et al., 2013, Bauer et al., 2015
384	*Symphyotrichum pilosum*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Pendergast et al., 2013
385	*Taeniatherum caput-medusae*	Poaceae	Grass	Annual	Grassland	Microbial shift	Blank and Sforza 2007
386	*Tanacetum vulgare*	Asteraceae	Forb	Perennial	Grassland	Microbial shift/Soilborne pathogen	Schittko et al., 2016, Petermann et al., 2008, Schittko and Wurst 2014

387	*Taraxacum officinale*	Asteraceae	Forb	Perennial	Grassland	Microbial shift/Autotoxicity/ Nutrient imbalance or depletion	Klironomos 2002, Cortois et al., 2016, Chiuffo et al., 2015, Bergmann et al., 2016, Anacker et al., 2014
388	*Thelesperma megapotamicum*	Asteraceae	Forb	Perennial	Semiarid/arid	Microbial shift	Chiuffo et al., 2015
389	*Thymus capitatus*	Lamiaceae	Tree/Shrub	Perennial	Grassland	Autotoxicity	Vokou and Margaris 1986
390	*Tilia americana*	Polygonaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Bennett et al., 2017
391	*Tragopogon dubius*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Van Grunsven et al., 2007, Chiuffo et al., 2015
392	*Tragopogon dubius*	Asteraceae	Forb	Perennial	Semiarid/arid	Microbial shift	Chiuffo et al., 2015
393	*Tragopogon pratensis*	Asteraceae	Forb	Perennial	Grassland	Microbial shift	Van Grunsven et al., 2007, Klironomos 2002, Cortois et al., 2016, Anacker et al., 2014
394	*Trifolium campestre*	Fabaceae	Forb	Perennial	Grassland	Microbial shift/Soilborne pathogen	Petermann et al., 2008, Cortois et al., 2016
395	*Trifolium dubium*	Fabaceae	Forb	Perennial	Grassland	Microbial shift	Cortois et al., 2016
396	*Trifolium hybridum*	Fabaceae	Forb	Perennial	Grassland	Microbial shift	Cortois et al., 2016
397	*Trifolium incarnatum*	Fabaceae	Forb	Perennial	Grassland	Soilborne pathogen	Petermann et al., 2008
398	*Trifolium pratense*	Fabaceae	Forb	Perennial	Grassland	Microbial shift/Soilborne Pathogen/Nutrient imbalance or depletion	Wubs and Bezemer 2016, Wagg et al., 2015, Petermann et al., 2008, Klironomos 2002, Fitzpatrick et al., 2016, Cortois et al., 2016, Bartlet-Ryser et al. 2005, Anacker et al., 2014
399	*Trifolium repens*	Fabaceae	Forb	Perennial	Grassland	Microbial shift/Soilborne pathogen/Autotoxicity	Zuppinger-Dingley et al., 2016, Schittko et al., 2016, Petermann et al., 2008, Newman and Rovira 1975, García-Parisi and Omacini 2017, Cortois et al., 2016
400	*Trisetum flavescens*	Poaceae	Grass	Annual	Grassland	Microbial shift	Cortois et al., 2016
401	*Tsuga canadensis*	Pinaceae	Tree/Shrub	Perennial	Boreal forest	Microbial shift	Kotanen 2007, Bennett et al., 2017

402	*Tsuga mertensiana*	Pinaceae	Tree/Shrub	Perennial	Boreal forest	Soilborne pathogen/Nutrient imbalance or depletion	Matson and Boone 1984
403	*Typha latifolia*	Typhaceae	Forb	Perennial	Wetlands	Microbial shift/Autotoxicity	McNaughton 1968, Grace 1983
404	*Verbesina encelioides*	Asteraceae	Forb	Perennial	Semiarid/arid	Microbial shift	Chiuffo et al., 2015
405	*Veronica chamaedrys*	Scrophulariaceae	Forb	Perennial	Grassland	Microbial shift	Cortois et al., 2016
406	*Veronica officinalis*	Scrophulariaceae	Forb	Perennial	Grassland	Microbial shift	Klironomos 2002, Anacker et al., 2014
407	*Viburnum dentatum*	Adoxaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Elgersma et al., 2012
408	*Vicia cracca*	Fabaceae	Forb	Annual	Grassland	Microbial shift/Soilborne pathogen	Petermann et al., 2008, Klironomos 2002, Cortois et al., 2016, Anacker et al., 2014
409	*Vicia villosa*	Fabaceae	Forb	Annual	Grassland	Soilborne pathogen	Petermann et al., 2008
410	*Vincetoxicum rossicum*	Apocynaceae	Vine	Perennial	Grassland	Nutrient imbalance or depletion	Sanderson et al., 2015
411	*Viola arvensis*	Violaceae	Forb	Annual	Grassland	Microbial shift	Kardol et al., 2007
412	*Virola koschnyi*	Myristicaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	McCarthy-Neumann and Kobe 2010b
413	*Vulpia ciliata*	Poaceae	Grass	Annual	Grassland	Microbial shift/Soilborne pathogen	Van der Putten et al., 2001
414	*Wollemia nobilis*	Araucariaceae	Tree/Shrub	Perennial	Temperate forest	Microbial shift	Rigg et al., 2016
415	*Zizania palustris*	Poaceae	Grass	Annual	Wetlands	Nutrient imbalance or depletion	Walker et al., 2006

References to Annex Table 1

Abdul-Wahab, A. S. & Rice, E. L. (1967). Plant inhibition by Johnson grass and its possible significance in old-field succession. *Bulletin of the Torrey Botanical Club*, 486-497.

Agrawal, A. A., Kotanen, P. M., Mitchell, C. E., Power, A. G., Godsoe, W. & Klironomos, J. (2005). Enemy release? An experiment with congeneric plant pairs and diverse above-and belowground enemies. *Ecology,* **86,** 2979-2989.

Anacker, B. L., Klironomos, J. N., Maherali, H., Reinhart, K. O. & Strauss, S. Y. (2014). Phylogenetic conservatism in plant-soil feedback and its implications for plant abundance. *Ecology letters,* **17,** 1613-1621.

Anaya, A. L. & Amo, S. (1978). Allelopathic potential ofAmbrosia cumanensis HBK (Compositae) in a tropical zone of Mexico. *Journal of Chemical Ecology,* **4,** 289-304.

Armstrong, J. & Armstrong, W. (2001). Rice and Phragmites: effects of organic acids on growth, root permeability, and radial oxygen loss to the rhizosphere. *American Journal of Botany,* **88,** 1359-1370.

Ballegaard, T. & Warncke, E. (1985). The age distribution of a Cirsium palustre population in a spring area in Jutland, Denmark. *Ecography,* **8,** 59-62.

Batten, K. M., Scow, K. M. & Espeland, E. K. (2008). Soil microbial community associated with an invasive grass differentially impacts native plant performance. *Microbial Ecology,* **55,** 220-228.

Bauer, J. T., Mack, K. M. & Bever, J. D. (2015). Plant-soil feedbacks as drivers of succession: evidence from remnant and restored tallgrass prairies. *Ecosphere,* **6,** 1-12.

Bayandala, B., Fukasawa, Y. & Seiwa, K. (2016). Roles of pathogens on replacement of tree seedlings in heterogeneous light environments in a temperate forest: a reciprocal seed sowing experiment. *Journal of Ecology*.

Beckstead, J. & Parker, I. M. (2003). Invasiveness of ammophila arenaria: release from soil-borne pathogens? *Ecology,* **84,** 2824-2831.

Belnap, J., Phillips, S. L., Sherrod, S. K. & Moldenke, A. (2005). Soil biota can change after exotic plant invasion: does this affect ecosystem processes? *Ecology,* **86,** 3007-3017.

Bennett, J. A., Maherali, H., Reinhart, K. O., Lekberg, Y., Hart, M. M. & Klironomos, J. (2017). Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science,* **355,** 181-184.

Bergmann, J., Verbruggen, E., Heinze, J., Xiang, D., Chen, B., Joshi, J. & Rillig, M. C. (2016). The interplay between soil structure, roots, and microbiota as a determinant of plant–soil feedback. *Ecology and Evolution,* **6,** 7633-7644.

Bevege, D. (1968). Inhibition of seedling hoop pine (Araucaria cunninghamii Ait.) on forest soils by phytotoxic substances from the root zones of Pinus, Araucaria and Flindersia. *Plant and soil,* **29,** 263-273.

Bever, J. D. (1994). Feeback between plants and their soil communities in an old field community. *Ecology,* **75,** 1965-1977.

Bezemer, T., Lawson, C. S., Hedlund, K., Edwards, A. R., Brook, A. J., Igual, J. M., Mortimer, S. R. & Van Der Putten, W. H. (2006a). Plant species and functional group effects on abiotic and microbial soil properties and plant–soil feedback responses in two grasslands. *Journal of Ecology,* **94,** 893-904.

Bezemer, T. M., Harvey, J. A., Kowalchuk, G. A., Korpershoek, H. & van der Putten, W. H. (2006b). Interplay between Senecio jacobaea and plant, soil, and aboveground insect community composition. *Ecology,* **87,** 2002-2013.

Blank, R. R. & Sforza, R. (2007). Plant–soil relationships of the invasive annual grass Taeniatherum caput-medusae: a reciprocal transplant experiment. *Plant and soil,* **298,** 7-19.

Bodelier, P. L., Stomp, M., Santamaria, L., Klaassen, M. & Laanbroek, H. J. (2006). Animal–plant–microbe interactions: direct and indirect effects of swan foraging behaviour modulate methane cycling in temperate shallow wetlands. *Oecologia,* **149,** 233-244.

Bokhari, U. (1978). Allelopathy among prairie grasses and its possible ecological significance. *Annals of Botany,* **42,** 127-136.

Bonanomi, G. & Allegrezza, M. (2004). Effetti della colonizzazione di Brachypodium rupestre (Host) Roemer et Schultes sulla diversità di alcune fitocenosi erbacee dell'Appennino centrale. *Fitosociologia,* **41,** 51-69.

Bonanomi, G., Legg, C. & Mazzoleni, S. (2005a). Autoinhibition of germination and seedling establishment by leachate of Calluna vulgaris leaves and litter. *Community Ecology,* **6,** 203-208.

Bonanomi, G. & Mazzoleni, S. (2005). Soil history affects plant growth and competitive ability in herbaceous species. *Community Ecology,* **6,** 23-28.

Bonanomi, G., Rietkerk, M., Dekker, S. C. & Mazzoleni, S. (2005b). Negative plant–soil feedback and positive species interaction in a herbaceous plant community. *Plant ecology,* **181,** 269.

Bonanomi, G., Rietkerk, M., Dekker, S. C. & Mazzoleni, S. (2007). Islands of fertility induce co-occurring negative and positive plant-soil feedbacks promoting coexistence.

Bradow, J. M. & Connick, W. J. (1988). Seed-germination inhibition by volatile alcohols and other compounds associated withAmaranthus palmeri residues. *Journal of Chemical Ecology,* **14,** 1633-1648.

Bukowski, A. R. & Petermann, J. S. (2014). Intraspecific plant–soil feedback and intraspecific overyielding in Arabidopsis thaliana. *Ecology and Evolution,* **4,** 2533-2545.

Burns, J. H., Brandt, A. J., Murphy, J. E., Kaczowka, A. M. & Burke, D. J. (2017). Spatial heterogeneity of plant–soil feedbacks increases per capita reproductive biomass of species at an establishment disadvantage. *Oecologia,* **183,** 1077-1086.

Callaway, R. M., Thelen, G. C., Barth, S., Ramsey, P. W. & Gannon, J. E. (2004). Soil fungi alter interactions between the invader Centaurea maculosa and North American natives. *Ecology,* **85,** 1062-1071.

Canals, R., Emeterio, L. & Peralta, J. (2005). Autotoxicity in Lolium rigidum: analyzing the role of chemically mediated interactions in annual plant populations. *Journal of Theoretical Biology,* **235,** 402-407.

Casper, B. B., Bentivenga, S. P., Ji, B., Doherty, J. H., Edenborn, H. M. & Gustafson, D. J. (2008). Plant–soil feedback: testing the generality with the same grasses in serpentine and prairie soils. *Ecology,* **89,** 2154-2164.

Casper, B. B. & Castelli, J. P. (2007). Evaluating plant–soil feedback together with competition in a serpentine grassland. *Ecology letters,* **10,** 394-400.

Castelli, J. P. & Casper, B. B. (2003). Intraspecific AM fungal variation contributes to plant–fungal feedback in a serpentine grassland. *Ecology,* **84,** 323-336.

Chapin, F. S., Walker, L. R., Fastie, C. L. & Sharman, L. C. (1994). Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. *Ecological Monographs,* **64,** 149-175.

Chen, L., Wang, S. & Yu, X. (2005). Effects of phenolics on seedling growth and 15N nitrate absorption of Cunninghamia lanceolata. *Allelopathy J,* **15,** 57-66.

Chiuffo, M. C., MacDougall, A. S. & Hierro, J. L. (2015). Native and non-native ruderals experience similar plant–soil feedbacks and neighbor effects in a system where they coexist. *Oecologia,* **179,** 843-852.

Chu-Chou, M. (1978). Effects of root residues on growth ofPinus radiata seedlings and a mycorrhizal fungus. *Annals of Applied Biology,* **90,** 407-416.

Chung, Y. A. & Rudgers, J. A. (2016). Plant–soil feedbacks promote negative frequency dependence in the coexistence of two aridland grasses. In: Proc. R. Soc. B. Pp. 20160608. The Royal Society.

Cortois, R., Schröder-Georgi, T., Weigelt, A., Putten, W. H. & De Deyn, G. B. (2016). Plant–soil feedbacks: role of plant functional group and plant traits. *Journal of Ecology,* **104,** 1608-1617.

Crawford, K. M. & Knight, T. M. (2016). Competition overwhelms the positive plant–soil feedback generated by an invasive plant. *Oecologia***,** 1-10.

Curtis, J. & Cottam, G. (1950). Antibiotic and autotoxic effects in prairie sunflower. *Bulletin of the Torrey Botanical Club,* **77,** 187-191.

Danin, A. (1997). Shootborne Roots—An Adaptive Organ for Plants of Sand Dunes. In: Biology of Root Formation and Development. Pp. 221-226. Springer.

De Deyn, G. B., Raaijmakers, C. E., Van Ruijven, J., Berendse, F. & Van Der Putten, W. H. (2004). Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. *Oikos,* **106,** 576-586.

De Jong, T. J. & Klinkhamer, P. G. (1985). The negative effects of litter of parent plants of Cirsium vulgare on their offspring: autotoxicity or immobilization? *Oecologia,* **65,** 153-160.

Dickie, I. A., Montgomery, R. A., Reich, P. B. & Schnitzer, S. A. (2007). Physiological and phenological responses of oak seedlings to oak forest soil in the absence of trees. *Tree Physiology-Victoria-,* **27,** 133.

Dostálek, T., Münzbergová, Z., Kladivová, A. & Macel, M. (2016). Plant–soil feedback in native vs. invasive populations of a range expanding plant. *Plant and soil,* **399,** 209-220.

Durkee Walker, R., Pastor, J. & Dewey, B. W. (2006). Effects of wild rice (Zizania palustris) straw on biomass and seed production in northern Minnesota. *Botany,* **84,** 1019-1024.

Edwards, C. A. & Fletcher, K. (1988). Interactions between earthworms and microorganisms in organic-matter breakdown. *Agriculture, Ecosystems & Environment,* **24,** 235-247.

Ehlers, B. K. & Thompson, J. (2004). Do co-occurring plant species adapt to one another? The response of Bromus erectus to the presence of different Thymus vulgaris chemotypes. *Oecologia,* **141,** 511-518.

Elgersma, K. J., Yu, S., Vor, T. & Ehrenfeld, J. G. (2012). Microbial-mediated feedbacks of leaf litter on invasive plant growth and interspecific competition. *Plant and soil,* **356,** 341-355.

Ervin, G. N. & Wetzel, R. G. (2000). Allelochemical autotoxicity in the emergent wetland macrophyte Juncus effusus (Juncaceae). *American Journal of Botany,* **87,** 853-860.

Fitzpatrick, C. R., Gehant, L., Kotanen, P. M. & Johnson, M. T. (2016). Phylogenetic relatedness, phenotypic similarity, and plant-soil feedbacks. *Journal of Ecology*.

Fitzsimons, M. S. & Miller, R. M. (2010). The importance of soil microorganisms for maintaining diverse plant communities in tallgrass prairie. *American Journal of Botany,* **97,** 1937-1943.

Florence, R. (1965). Decline of Old-Growth Redwood Forests in Relation to Some Soil Microbiological Processes. *Ecology,* **46,** 52-64.

Florence, R. & Crocker, R. (1962). Analysis of blackbutt (Eucalyptus pilularis Sm.) seedling growth in a blackbutt forest soil. *Ecology,* **43,** 670-679.

Friedman, J., Rushkin, E. & Waller, G. R. (1982). Highly potent germination inhibitors in aqueous eluate of fruits of bishop's weed (Ammi majus L.) and avoidance of autoinhibition. *Journal of Chemical Ecology,* **8,** 55-65.

Frouz, J., Toyota, A., Mudrák, O., Jílková, V., Filipová, A. & Cajthaml, T. (2016). Effects of soil substrate quality, microbial diversity and community composition on the plant community during primary succession. *Soil Biology and Biochemistry,* **99,** 75-84.

García-Parisi, P. & Omacini, M. (2017). Arbuscular mycorrhizal fungi can shift plant-soil feedback of grass-endophyte symbiosis from negative to positive. *Plant and soil***,** 1-11.

Gillespie, I. G. & Allen, E. B. (2006). Effects of soil and mycorrhizae from native and invaded vegetation on a rare California forb. *Applied Soil Ecology,* **32,** 6-12.

Gopal, B. & Goel, U. (1993). Competition and allelopathy in aquatic plant communities. *The Botanical Review,* **59,** 155-210.

Grace, J. B. (1983). Autotoxic inhibition of seed germination by Typha latifolia: an evaluation. *Oecologia,* **59,** 366-369.

Grant, E. & Sallans, W. (1964). Influence of plant extracts on germination and growth of eight forage species. *Grass and Forage Science,* **19,** 191-197.

Groner, M. G. (1974). Intraspecific allelopathy in Kalanchoe daigremontiana. *Botanical Gazette,* **135,** 73-79.

Gundale, M. J., Kardol, P., Nilsson, M. C., Nilsson, U., Lucas, R. W. & Wardle, D. A. (2014). Interactions with soil biota shift from negative to positive when a tree species is moved outside its native range. *New Phytologist,* **202,** 415-421.

Gustafson, D. J. & Casper, B. B. (2004). Nutrient addition affects AM fungal performance and expression of plant/fungal feedback in three serpentine grasses. *Plant and soil,* **259,** 9-17.

Harborne, J. B. (1972). Phytochemical ecology. *Phytochemical ecology.*

Hegazy, A. K., Mansour, K. S. & Abdel-Hady, N. F. (1990). Allelopathic and autotoxic effects ofAnastatica hierochuntica L. *Journal of Chemical Ecology,* **16,** 2183-2193.

Heinze, J., Sitte, M., Schindhelm, A., Wright, J. & Joshi, J. (2016). Plant-soil feedbacks: a comparative study on the relative importance of soil feedbacks in the greenhouse versus the field. *Oecologia,* **181,** 559-569.

Hemrová, L., Knappová, J. & Münzbergová, Z. (2016). Assessment of habitat suitability is affected by plant-soil feedback: comparison of field and garden experiment. *PloS one,* **11,** e0157800.

Hendriks, M., Mommer, L., Caluwe, H., Smit-Tiekstra, A. E., Putten, W. H. & Kroon, H. (2013). Independent variations of plant and soil mixtures reveal soil feedback effects on plant community overyielding. *Journal of Ecology,* **101,** 287-297.

Holah, J. C. & Alexander, H. M. (1999). Soil pathogenic fungi have the potential to affect the co-existence of two tallgrass prairie species. *Journal of Ecology,* **87,** 598-608.

Hood, L., Heath, J. R., Phelps, M. E. & Lin, B. (2004). Systems biology and new technologies enable predictive and preventative medicine. *Science,* **306,** 640-643.

Jiang, L., Han, X., Zhang, G. & Kardol, P. (2010). The role of plant–soil feedbacks and land-use legacies in restoration of a temperate steppe in northern China. *Ecological research,* **25,** 1101-1111.

Jing, J., Bezemer, T. M. & Putten, W. H. (2015). Complementarity and selection effects in early and mid-successional plant communities are differentially affected by plant–soil feedback. *Journal of Ecology,* **103,** 641-647.

Jordan, N. R., Larson, D. L. & Huerd, S. C. (2008). Soil modification by invasive plants: effects on native and invasive species of mixed-grass prairies. *Biological invasions,* **10,** 177-190.

Kardol, P., Cornips, N. J., van Kempen, M. M., Bakx-Schotman, J. & van der Putten, W. H. (2007). Microbe-mediated plant–soil feedback causes historical contingency effects in plant community assembly. *Ecological Monographs,* **77,** 147-162.

Keever, C. (1950). Causes of succession on old fields of the Piedmont, North Carolina. *Ecological Monographs,* **20,** 229-250.

Keymer, D. P. & Lankau, R. A. (2016). Disruption of plant-soil-microbial relationships influences plant growth. *Journal of Ecology*.

Kiers, E. T., Lovelock, C. E., Krueger, E. L. & Herre, E. A. (2000). Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. *Ecology letters,* **3,** 106-113.

King, D. C. (2015). Plant-Soil Feedbacks of the Invasive Centaurea Solstitialis.

Klironomos, J. N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature,* **417,** 67-70.

Knevel, I. C., Lans, T., Menting, F. B., Hertling, U. M. & van der Putten, W. H. (2004). Release from native root herbivores and biotic resistance by soil pathogens in a new habitat both affect the alien Ammophila arenaria in South Africa. *Oecologia,* **141,** 502-510.

Koppel, J. v. d. & Crain, C. M. (2006). Scale-dependent inhibition drives regular tussock spacing in a freshwater marsh. *The American Naturalist,* **168,** E136-E147.

Kos, M., Tuijl, M. A., de Roo, J., Mulder, P. P. & Bezemer, T. M. (2015a). Plant–soil feedback effects on plant quality and performance of an aboveground herbivore interact with fertilisation. *Oikos,* **124,** 658-667.

Kos, M., Tuijl, M. A., Roo, J., Mulder, P. P. & Bezemer, T. M. (2015b). Species-specific plant–soil feedback effects on above-ground plant–insect interactions. *Journal of Ecology,* **103,** 904-914.

Kotanen, P. M. (2007). Effects of fungal seed pathogens under conspecific and heterospecific trees in a temperate forest. *Botany,* **85,** 918-925.

Kuebbing, S. E., Classen, A. T., Call, J. J., Henning, J. A. & Simberloff, D. (2015). Plant–soil interactions promote co-occurrence of three nonnative woody shrubs. *Ecology,* **96,** 2289-2299.

Kueffer, C., Kronauer, L. & Edwards, P. J. (2009). Wider spectrum of fruit traits in invasive than native floras may increase the vulnerability of oceanic islands to plant invasions. *Oikos,* **118,** 1327-1334.

Kulmatiski, A., Beard, K. H., Grenzer, J., Forero, L. & Heavilin, J. (2016). Using plant-soil feedbacks to predict plant biomass in diverse communities. *Ecology,* **97,** 2064-2073.

Larios, L. & Suding, K. N. (2015). Competition and soil resource environment alter plant–soil feedbacks for native and exotic grasses. *AoB Plants,* **7,** plu077.

Li, J. & Romane, F. J. (1997). Effects of germination inhibition on the dynamics of Quercus ilex stands. *Journal of Vegetation Science,* **8,** 287-294.

Liang, M., Liu, X., Gilbert, G. S., Zheng, Y., Luo, S., Huang, F. & Yu, S. (2016). Adult trees cause density-dependent mortality in conspecific seedlings by regulating the frequency of pathogenic soil fungi. *Ecology letters,* **19,** 1448-1456.

Lodhi, M. (1979a). Allelopathic potential ofSalsola kali L. and its possible role in rapid disappearance of weedy stage during revegetation. *Journal of Chemical Ecology,* **5,** 429-437.

Lodhi, M. (1979b). Germination and decreased growth of Kochia scoparia in relation to its autoallelopathy. *Canadian Journal of Botany,* **57,** 1083-1088.

Lou, Y., Clay, S. A., Davis, A. S., Dille, A., Felix, J., Ramirez, A. H., Sprague, C. L. & Yannarell, A. C. (2014). An affinity–effect relationship for microbial communities in plant–soil feedback loops. *Microbial Ecology,* **67,** 866-876.

Mallik, A. & Newton, P. (1988). Inhibition of black spruce seedling growth by forest-floor substrates of central Newfoundland. *Forest ecology and management,* **23,** 273-283.

Maron, J. L., Klironomos, J., Waller, L. & Callaway, R. M. (2014). Invasive plants escape from suppressive soil biota at regional scales. *Journal of Ecology,* **102,** 19-27.

Maron, J. L., Laney Smith, A., Ortega, Y. K., Pearson, D. E. & Callaway, R. M. (2016). Negative plant-soil feedbacks increase with plant abundance, and are unchanged by competition. *Ecology,* **97,** 2055-2063.

Matson, P. A. & Boone, R. D. (1984). Natural Disturbance and Nitrogen Mineralization: Wave-Form Dieback of Mountain Hemlock in the Oregon Cascades. *Ecology,* **65,** 1511-1516.

Mazzoleni, S., Bonanomi, G., Giannino, F., Rietkerk, M., Dekker, S. & Zucconi, F. (2007). Is plant biodiversity driven by decomposition processes? An emerging new theory on plant diversity. *Community Ecology,* **8,** 103-109.

Mazzoleni, S., Bonanomi, G., Incerti, G., Chiusano, M. L., Termolino, P., Mingo, A., Senatore, M., Giannino, F., Cartenì, F. & Rietkerk, M. (2015). Inhibitory and toxic effects of extracellular self-DNA in litter: a mechanism for negative plant–soil feedbacks? *New Phytologist,* **205,** 1195-1210.

McCarthy-Neumann, S. & Kobe, R. K. (2010a). Conspecific and heterospecific plant–soil feedbacks influence survivorship and growth of temperate tree seedlings. *Journal of Ecology,* **98,** 408-418.

McCarthy-Neumann, S. & Kobe, R. K. (2010b). Conspecific plant–soil feedbacks reduce survivorship and growth of tropical tree seedlings. *Journal of Ecology,* **98,** 396-407.

McNaughton, S. (1968). Autotoxic Feedback in Relatin to Germination and Seedling Growth in Typha Latifolia. *Ecology,* **49,** 367-369.

Meiman, P. J., Redente, E. F. & Paschke, M. W. (2006). The role of the native soil community in the invasion ecology of spotted (Centaurea maculosa auct. non Lam.) and diffuse (Centaurea diffusa Lam.) knapweed. *Applied Soil Ecology,* **32,** 77-88.

Miller, D. A. (1996). Allelopathy in forage crop systems. *Agronomy Journal,* **88,** 854-859.

Molofsky, J., Lanza, J. & Crone, E. E. (2000). Plant litter feedback and population dynamics in an annual plant, Cardamine pensylvanica. *Oecologia,* **124,** 522-528.

Moral, R. D. & Cates, R. G. (1971). Allelopathic potential of the dominant vegetation of western Washington. *Ecology,* **52,** 1030-1037.

Morris, C., Call, C. A., Monaco, T. A., Grossl, P. R. & Dewey, S. A. (2006). Evaluation of elemental allelopathy in Acroptilon repens (L.) DC.(Russian Knapweed). *Plant and soil,* **289,** 279-288.

Nagendra, U. J. & Peterson, C. J. (2016). Plant-soil feedbacks differ in intact and tornado-damaged areas of the southern Appalachian mountains, USA. *Plant and soil,* **402,** 103-116.

Newman, E. & Rovira, A. (1975). Allelopathy among some British grassland species. *The Journal of Ecology***,** 727-737.

Nijjer, S., Rogers, W. E. & Siemann, E. (2007). Negative plant–soil feedbacks may limit persistence of an invasive tree due to rapid accumulation of soil pathogens. *Proceedings of the Royal Society of London B: Biological Sciences,* **274,** 2621-2627.

Niu, H.-b., Liu, W.-x., Wan, F.-h. & Liu, B. (2007). An invasive aster (Ageratina adenophora) invades and dominates forest understories in China: altered soil microbial communities facilitate the invader and inhibit natives. *Plant and soil,* **294,** 73-85.

Olff, H., Hoorens, B., De Goede, R., Van der Putten, W. & Gleichman, J. (2000). Small-scale shifting mosaics of two dominant grassland species: the possible role of soil-borne pathogens. *Oecologia,* **125,** 45-54.

Oremus, P. & Otten, H. (1983). Nematodes (Longidorus n. sp. and Tylenchorhynchus microphasmis Loof) in growth and nodulation of Sea Buckthorn (Hippophaë rhamnoides L.). *Plant Soil,* **73,** 141-147.

Packer, A. & Clay, K. (2000). Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature,* **404,** 278-281.

Parenti, R. L. & Rice, E. L. (1969). Inhibitional effects of Digitaria sanguinalis and possible role in old-field succession. *Bulletin of the Torrey Botanical Club***,** 70-78.

Pellissier, F. & Souto, X. C. (1999). Allelopathy in northern temperate and boreal semi-natural woodland. *Critical Reviews in Plant Sciences,* **18,** 637-652.

Pendergast, T. H., Burke, D. J. & Carson, W. P. (2013). Belowground biotic complexity drives aboveground dynamics: a test of the soil community feedback model. *New Phytologist,* **197,** 1300-1310.

Perkins, L. B. & Nowak, R. S. (2012). Soil conditioning and plant–soil feedbacks affect competitive relationships between native and invasive grasses. *Plant ecology,* **213,** 1337-1344.

Perry, L. G., Thelen, G. C., Ridenour, W. M., Weir, T. L., Callaway, R. M., Paschke, M. W. & M VIVANCO, J. (2005). Dual role for an allelochemical:(±)-catechin from Centaurea maculosa root exudates regulates conspecific seedling establishment. *Journal of Ecology,* **93,** 1126-1135.

Petermann, J. S., Fergus, A. J., Turnbull, L. A. & Schmid, B. (2008). Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology,* **89,** 2399-2406.

Picman, J. & Picman, A. K. (1984). Autotoxicity in Parthenium hysterophorus and its possible role in control of germination. *Biochemical Systematics and Ecology,* **12,** 287-292.

Puerta-Piñero, C., Gómez, J. M. & Zamora, R. (2006). Species-specific effects on topsoil development affect Quercus ilex seedling performance. *Acta Oecologica,* **29,** 65-71.

Reinhart, K. O. & Callaway, R. M. (2004). Soil biota facilitate exotic Acer invasions in Europe and North America. *Ecological Applications,* **14,** 1737-1745.

Reinhart, K. O., Greene, E. & Callaway, R. M. (2005). Effects of Acer platanoides invasion on understory plant communities and tree regeneration in the northern Rocky Mountains. *Ecography,* **28,** 573-582.

Reinhart, K. O., Packer, A., Van der Putten, W. H. & Clay, K. (2003). Plant–soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology letters,* **6,** 1046-1050.

Rice, E. (1984). Allelopathy. Physiological Ecology. Orlando, FL, USA: Academic Press.

Rigg, J. L., Offord, C. A., Singh, B. K., Anderson, I., Clarke, S. & Powell, J. R. (2016). Soil microbial communities influence seedling growth of a rare conifer independent of plant–soil feedback. *Ecology,* **97,** 3346-3358.

Rutten, G., Prati, D., Hemp, A. & Fischer, M. (2016). Plant–soil feedback in East-African savanna trees. *Ecology,* **97,** 294-301.

Sanderson, L. A., Day, N. J. & Antunes, P. M. (2015). Edaphic factors and feedback do not limit range expansion of an exotic invasive plant. *Plant ecology,* **216,** 133-141.

Schittko, C., Runge, C., Strupp, M., Wolff, S. & Wurst, S. (2016). No evidence that plant–soil feedback effects of native and invasive plant species under glasshouse conditions are reflected in the field. *Journal of Ecology,* **104,** 1243-1249.

Schittko, C. & Wurst, S. (2014). Above-and belowground effects of plant-soil feedback from exotic Solidago canadensis on native Tanacetum vulgare. *Biological invasions,* **16,** 1465-1479.

Shannon, S. M., Bauer, J. T., Anderson, W. E. & Reynolds, H. L. (2014). Plant-soil feedbacks between invasive shrubs and native forest understory species lead to shifts in the abundance of mycorrhizal fungi. *Plant and soil,* **382,** 317-328.

Sigüenza, C., Corkidi, L. & Allen, E. B. (2006). Feedbacks of soil inoculum of mycorrhizal fungi altered by N deposition on the growth of a native shrub and an invasive annual grass. *Plant and soil,* **286,** 153-165.

Smith, L. M. & Reynolds, H. L. (2015). Plant–soil feedbacks shift from negative to positive with decreasing light in forest understory species. *Ecology,* **96,** 2523-2532.

Solomon, B. P. (1983). Autoallelopathy in Solanum carolinense: reversible delayed germination. *American Midland Naturalist***,** 412-418.

Stanescu, S. & Maherali, H. (2016). Arbuscular mycorrhizal fungi alter the competitive hierarchy among old-field plant species. *Oecologia***,** 1-13.

Suding, K. N., Gross, K. L. & Houseman, G. R. (2004). Alternative states and positive feedbacks in restoration ecology. *Trends in Ecology & Evolution,* **19,** 46-53.

Te Beest, M., Stevens, N., Olff, H. & Van Der Putten, W. H. (2009). Plant–soil feedback induces shifts in biomass allocation in the invasive plant Chromolaena odorata. *Journal of Ecology,* **97,** 1281-1290.

Tilman, D. & Wedin, D. (1991). Dynamics of nitrogen competition between successional grasses. *Ecology,* **72,** 1038-1049.

Troelstra, S., Wagenaar, R., Smant, W. & Peters, B. (2001). Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. *New Phytologist,* **150,** 697-706.

Valliere, J. M. & Allen, E. B. (2016). Interactive effects of nitrogen deposition and drought-stress on plant-soil feedbacks of Artemisia californica. *Plant and soil,* **403,** 277-290.

Van der Putten, W., Kowalchuk, G., Brinkman, E., Doodeman, G., Van der Kaaij, R., Kamp, A., Menting, F. & Veenendaal, E. (2007). Soil feedback of exotic savanna grass relates to pathogen absence and mycorrhizal selectivity. *Ecology,* **88,** 978-988.

Van der Putten, W., Van Dijk, C. & Peters, B. (1993). Plant-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature,* **362,** 53-56.

Van der Putten, W. H., Vet, L. E., Harvey, J. A. & Wäckers, F. L. (2001). Linking above-and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. *Trends in Ecology & Evolution,* **16,** 547-554.

Van Grunsven, R. H., Van Der Putten, W. H., Bezemer, T., Tamis, W. L., Berendse, F. & Veenendaal, E. M. (2007). Reduced plant–soil feedback of plant species expanding their range as compared to natives. *Journal of Ecology,* **95,** 1050-1057.

Vokou, D. & Margaris, N. (1986). Autoallelopathy of Thymus capitatus. *ACTA OECOL.(OECOL. PLANT.).* **7,** 157-163.

Wagg, C., Boller, B., Schneider, S., Widmer, F. & van der Heijden, M. G. (2015). Intraspecific and intergenerational differences in plant–soil feedbacks. *Oikos,* **124,** 994-1004.

Warrag, M. (1995). Autotoxic potential of foliage on seed germination and early growth of mesquite (Prosopis juliflora). *Journal of Arid Environments,* **31,** 415-421.

Webb, L., Tracey, J. & Haydock, K. (1967). A factor toxic to seedlings of the same species associated with living roots of the non-gregarious subtropical rain forest tree Grevillea robusta. *Journal of applied Ecology***,** 13-25.

Wedin, D. & Tilman, D. (1993). Competition among grasses along a nitrogen gradient: initial conditions and mechanisms of competition. *Ecological Monographs,* **63,** 199-229.

Wubs, E. & Bezemer, T. M. (2016). Effects of spatial plant–soil feedback heterogeneity on plant performance in monocultures. *Journal of Ecology,* **104,** 364-376.

Wurst, S., Kaiser, N., Nitzsche, S., Haase, J., Auge, H., Rillig, M. C. & Powell, J. R. (2015). Tree diversity modifies distance-dependent effects on seedling emergence but not plant–soil feedbacks of temperate trees. *Ecology,* **96,** 1529-1539.

Xia, Z. C., Kong, C. H., Chen, L. C., Wang, P. & Wang, S. L. (2016). A broadleaf species enhances an autotoxic conifers growth through belowground chemical interactions. *Ecology,* **97,** 2283-2292.

Yelenik, S. G. & Levine, J. M. (2011). The role of plant–soil feedbacks in driving native-species recovery. *Ecology,* **92,** 66-74.

Young, C. (1984). Autointoxication in root exudates ofAsparagus officinalis L. *Plant and soil,* **82,** 247-253.

Zhang, N., Van der Putten, W. H. & Veen, G. (2016). Effects of root decomposition on plant–soil feedback of early-and mid-successional plant species. *New Phytologist,* **212,** 220-231.

Zuppinger-Dingley, D., Flynn, D. F., De Deyn, G. B., Petermann, J. S. & Schmid, B. (2016). Plant selection and soil legacy enhance long-term biodiversity effects. *Ecology,* **97,** 918-928

Chapter 2

SOIL SICKNESS IN BABY LEAF CULTIVATION: AN EXPLORATIVE STUDY

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Abstract

Soil sickness is defined as a condition in which the constant use of non-sustainable agricultural practices such as monoculture, intensive tillage, constant application of chemical fertilizers and agrochemicals, cause a deterioration of soil physical, chemical and biological properties. As a consequence, negative effects on plant development and crop yields are observed. In the Piana del Sele, an agricultural area of Southern Italy with about 5,000 ha cultivated under greenhouses, the intensive cultivation system of baby leaf in the long period has led to an increase in soil sickness problems. In the present study, an explorative experiment was conducted to evaluate the spread of soil sickness in baby leaf monoculture, and to detect the main factors (e.g., nutritional, parasitic, toxic) involved in the reduction of crop yield. For this purpose, soil samples of twelve farms were subjected to different treatments, in order to selectively remove one or more of the proposed factors involved in soil sickness, and the efficacy on seed germination and growth of lettuce was evaluated. Compared with the untreated soil, autoclave-sterilized soil showed the highest percentage of seed germination, whereas the addition of mineral nutrients and soil sterilization with fumigants efficaciously promoted plant growth. The efficacy of soil treatments in improving plant growth in most of the sampled farms indicated that soil sickness of baby leaf is a common issue in the Piana del Sele. However, considering the heterogeneous responses among the applied treatments, further studies are required to elucidate the mechanisms and the role of biotic and abiotic factors involved in soil sickness.

1. Introduction

Soil sickness is a complex and multi-factorial phenomenon that appears when plants of the same species or its relatives are cultivated repeatedly on the same field, year after year i.e., monoculture (Zucconi, 2003). Consequently, negative interactions are established between plant and soil, leading to a reduction in both crop yield and quality, and a general decline of soil quality (Huang et al., 2013). The negative effects of soil sickness has been attributed to various abiotic and biotic factors, including soil nutrient depletion or imbalance (Bekunda, 1999; Howeler and Cadavid, 1990), build-up of soilborne pathogens and pests (Hartemink et al., 2000; Pankhurst et al., 2005), shifting in soil microbial community composition (Li et al., 2014; Nayyar et al., 2009), presence of autotoxic compounds (Asao et al., 2004; Singh et al., 1999), and accumulation of extracellular self-DNA (Mazzoleni et al., 2015).

The phenomenon of soil sickness was already known at the time of the ancient Greeks and Romans, but its spread was recorded in the second half of the 20th century as a result of the progress of intensive agriculture. This cultivation system is geared towards maximization of crop yields per unit of agricultural land area through the use of high-yielding crop varieties, repeated tillage, consecutive growth cycles and marked application of chemical fertilizers and agrochemicals (Zeng et al., 2008). In contrast, some traditional agricultural practices have been abandoned, including fallow, long-term crop rotation and application of organic amendments. Consequently, an increase in soil sickness was observed due to the deterioration of soil physical, chemical and biological properties (Huang et al., 2013; Wang et al., 2014; Zhou and Wu, 2015).

Problems of soil sickness are reported for several plant species cultivated both in open field and under protected environment, like greenhouse or plastic tunnel (Martínez et al., 2011; Minuto et al., 2002; Tagliavini and Marangoni, 1992). Cultivation under plastic tunnel gradually increased in the last decades and currently about 200,000 ha of the agricultural surface in the Mediterranean basin are covered using this system (Scarascia-Mugnozza et al., 2012). The main advantage is due to the improvement of microclimatic conditions that in turn allows a reduction of plant growth time and an increase of crop yield. However, this type of cultivation strongly affects soil quality by impacting on water, carbon and nutrient cycles. The permanent soil cover and the consequent request of localized irrigation to support crop water demand increases soil salinity, while the widespread use of synthetic mineral fertilizers can induce soil acidification (Ju et al., 2007). Moreover, the systematic elimination of crop residues, the frequent soil tillage and the optimal conditions of temperature and water content

can lead to a reduction of soil organic carbon content through mineralization process, with negative effects on soil physical-chemical quality and microbial community (Bonanomi et al., 2011). Also, the constant application of fumigants and other agrochemicals used to control plant pathogens, nematodes, pests and weeds, has a negative impact on soil quality and crop health because these compounds affect the composition of soil microbial community and the development of pesticide resistances (Tilman et al., 2002). Generally, the favorable conditions available under protected environment can produce a response in soil sickness greater than in the open field (Kulmatiski et al., 2008).

In the Piana del Sele, an agricultural area located in the Salerno province (Campania, Italy), more than 4,500 ha are covered with plastic tunnel, 3,000 of which are used for the cultivation of "baby leaf" (Fig. 1a-d). Economic market trends and the requirement of specialized machinery for crop production and harvesting have driven the farmers towards the cultivation of one or few vegetable species, mainly rocket (*Eruca sativa*) and lettuce (*Lactuca sativa*). The adoption of monoculture or short rotation, the high number of productive cycles per year and the frequent soil tillage led to excessive soil exploitation with consequent negative effects on the crops. Despite the application of mineral fertilizer to support plant nutrition and the use of fumigants and others agrochemicals to control plant diseases, an increase in soil sickness problems, like failure of seed germination, death of seedlings, stunted growth and yield reduction, were observed in the last years (Fig. 1e-f).

The present paper describes an explorative study concerning the soil sickness in baby leaf cultivation. For this purpose, soils from twelve farms located in the Piana del Sele were subject to different treatments in order to selectively remove one or more of the proposed mechanisms involved in soil sickness (i.e., nutritional, parasitic and toxic), and the efficacy of different soil treatments was evaluated on germination and growth of lettuce. The objectives of this study were: i) to evaluate the spread of soil sickness phenomenon in baby leaf monoculture; and ii) to detect the main factors involved in the reduction of crop yield.

2. Material and methods

2.1. Study area and soil collection

To evaluate the spread of soil sickness problem in baby leaf cultivation, twelve farms were selected in the Piana del Sele, a fertile alluvial plain located in Salerno province (Southern Italy). The study site has a Mediterranean climate, with hot and relatively dry summer (average temperature of 25.5°C in August) and mild and rainy winter (average temperature of 9.0°C in

January). Typically, rainfall reaches nearly 1,100 mm of rain every year, with a mean precipitation ranging from 105 mm in winter months to 25 mm in summer months.

The selected farms are specialized in the cultivation of baby leaf, mainly rocket and lettuce, that has been carried out under plastic tunnels for ~10 years. The protection structures are characterized by low-technology and unheated polyethylene-covered (height ~4 m). All farms adopted an intensive cultivation system based on repeated cultivation cycles (~5-6 cycles per year), frequent soil tillage, use of mineral fertilizers by fertigation system and soil disinfestation treatments.

In spring 2014, about 50 kg of soil for each farm were collected from the first 20 cm layer after removal of surface plant residues. The soil samples were packed in polyethylene bags, transferred at the greenhouse of the Department of Agricultural Science, Portici (40°48'46"N - 14°20'38"E), air dried at room temperature and sieved through a 2mm metal sieve.

2.2. Soil treatments

To investigate the mechanisms involved in soil sickness (i.e., nutritional, parasitic and toxic), the collected soils were subjected to six different treatments plus the untreated soil (US) as the control. Specifically, mineral fertilizer (MF), activated carbon (AC), autoclave sterilization (AS) and soil fumigation (SF) treatments were performed individually or in combination in order to exclude one or more of the proposed mechanisms (Table 1).

MF treatment was performed to exclude nutrient deficiency by adding Agromaster Balanced (15-7-15+2MgO+39SO_3) at a rate of 2 g/Kg soil, prior to sowing. Powder of AC, a form of processed carbon with a highly porous structure and high absorption capacity, was applied at 3% (w/w) to absorb putative organic toxic compounds present in the soil. To selectively remove pathogens and other deleterious microorganisms, physical and chemical soil sterilization treatment was performed by exposing the soils at high temperature (i.e., AS) or fumigant action (i.e., SF), respectively. For AS treatment, autoclavable bags were filled with moist soil from each farm and treated at 120 °C for 1 h to guarantee a complete sterilization until the centre of the bags. For SF treatment, soils were transferred into plastic bags, mixed with Basamid (200 mg/L soil), moistened to activate the chemical and incubated at room temperature for 2 weeks. At the end, the bags were opened and the soil was aired for 3 weeks to ensure a complete release of the chemical, then cress germination test was performed to verify the absence of phytotoxicity.

2.3. Pot experiment

Pot experiment was carried out to compare the seedling emergence and the shoot biomass of lettuce on soil of the twelve farms selected for the six soil treatments plus the untreated soil as the control. Sterilized round pots (diameter 14 cm, height 12 cm) were filled with 300 g of treated soil, then ten seeds of lettuce (*Lactuca sativa* L.) were sown in each pot, covered with a thin layer (~3 mm) of soil and watered to 80% of field capacity. The pots were placed in greenhouse equipped with automatic control of temperature (22 ± 4°C day and 16 ± 4°C night) and arranged in a completely randomized factorial design. A total of 420 pots were used with soils from the twelve sampled farms (hereafter named from Farm 1 to Farm 12), seven soil treatments and five replicates. During the experiment, pots were watered every 2-3 days to maintain soil moisture content between 60% and 80% of field capacity.

2.4. Evaluation of results and statistical analysis

Ten days after sowing, seeds germination was recorded by counting the number of seedlings emerged in each pot, then the number of plants was monitored once a week. At the end of the experiment, i.e. after 40 days of growth, plants of each pot were cut at the soil surface, and fresh shoot biomass was measured. For statistical analysis of the results, data were transformed to satisfy the assumptions of normality and homogeneity of variance, and submitted to analysis with the software STATISTICA 7. Data pertaining soil from the different farms were either analysed within each individual farm and after pooling data all the 12 farms. The effect of soil treatments on seedling emergence and shoot biomass were subjected to one-way ANOVA followed by Duncan's test (p=0.05).

3. Results

3.1. Seed germination

Seed germination was significantly affected by soil treatments, with the highest germination rate (~90%) observed for AS treatment (Fig. 2a). The other treatments showed a percentage of germination lower than US, with very low emergency for SF+AC+MF (Fig. 2a). In detail, when the results were analysed individually for each soil sample, AS treatment exhibited a significant improvement of seed germination in the soil from four farms, compared with the respective US (Fig. 3). The other treatments showed better germination rates than US only in the case of the soil of Farm 2, whereas no significant improvement or a lower seed germination was observed in the other cases (Fig. 3). As regards the mortality from germination

until the end of the experiment, no variation in the number of alive plants was observed (data not shown).

3.2. Plant growth

Differently from seeds germination, plants biomass exhibited a better response when soils were subject to different treatments. On average, MF and SF showed a significant increase in shoot biomass when compared with the US treatment, with mean fresh weight of 15.40 g/pot for MF and 13.22 g/pot for SF (Fig. 2b). When the responses of treatments in each farm were analysed individually, MF and SF showed a significant improvement of plant biomass in seven out of twelve soils, as compared with the respective US (Fig. 4). SF+AC and AS significantly increased the productivity in the soil of five and two farms, respectively, whereas the remaining (i.e., AC and SF+AC+MF) treatments exhibited a significant positive effect only in one case (Fig. 4).

4. Discussion

Soil sickness is a very complex phenomenon in which several factors, including nutrient imbalance or depletion, presence of autotoxic compounds, build-up of soilborne pathogens and deleterious microorganisms, can negatively affect germination, growth and crop productivity. In this study, soils of twelve farms in which baby leaf was consecutively cultivated for many years, underwent different treatments in order to disentangle the role of the different putative mechanisms involved in soil sickness (Table 1).

Generally, seed germination is influenced by environmental conditions and soil properties like temperature, water content, salinity, nitrate and ammonium concentration, presence of toxic compounds (allelopathic) and microbial community composition (Bewley and Black, 1994). An adequate level of nitrate dissolved in the soil can stimulate seed germination (Dyer, 1995), while the presence of allelopathic compounds, released directly by the plants through root exudates or by microorganisms during decomposition of organic material, may have an inhibitory effect depending on type and concentration of toxic compounds (Asao et al., 2004; He et al., 2009; Singh et al., 1999). In a study to evaluate the autotoxicity of tomato cultivated in hydroponic system, Yu et al. (1993) found that the culture solution collected at the end of the cultivation cycle strongly reduce the percentage of tomato seed germination. On the contrary, no inhibitory effect was observed in culture solution treated with activated carbon. Their results suggest both the presence of phytotoxic compounds released by root exudates and

the efficacy of activated carbon in the absorption of these compounds. In our study, both the application of mineral fertilizer (MF) and activated carbon (AC) showed a low efficacy in improving seed germination with respect to the control (US). On the contrary, a significant increase in the percentage of seed germination was found when soil had previously been sterilized in autoclave (AS). This result could suggest that microbial component is the principal responsible for the reduction of seed germination. However, chemical sterilization treatment, e.g. SF, as well as the combination with other treatments such as SF+AC and SF+AC+MF shown, on average, a lower germination percentage than US. Several authors reported that, when compared to soil fumigants, treatment with steam sterilization differently affects some biotic and abiotic soil properties like pH, concentration of ammonium and nitrate, availability of manganese, microbial community structure, enzymatic activities and, finally, the toxicity of some organic compounds (Chen et al., 1991; Tanaka et al., 2003; Yamamoto et al., 2008). Therefore, the highest seed germination found when the soil was disinfected with AS can be due to changes of both biotic and abiotic soil properties.

Unlike seed germination, the shoot biomass showed a positive response in MF and SF treated soils. Generally, MF and SF showed a significant increase in shoot biomass of 94% and 67%, respectively, when compared with control soil (US). On the contrary, no significant difference was observed for the remain other treatments. Mineral nutrition is essential for plant survival and growth because the adsorbed ions become part of many biochemical processes, including photosynthesis, enzymatic activity, cell functions, etc. (Engels et al., 2011). The deficiency in one or more minerals produces symptoms like chlorosis, necrosis, discoloration, and negatively affects plant growth and reproductivity (Marschner, 2012). In agroecosystem, evidence of soil sickness caused by nutrient depletion are scarce and refer to agronomic condition with very low input of organic or synthetic fertilizer (Bekunda, 1999; Howeler and Cadavid, 1990). In other cases, deficiency in plant nutrition can be due to an immobilization of nutrients in the soil. For instance, in continuous wetland rice cultivation, the anaerobic conditions lead to a slow degradation of the straw rice constituent, especially lignin, resulting in the formation of lignin-derived phenols compounds that accumulate in the soil and chemically immobilize N into stable compounds. As a result, the nitrogen supplied is locked in forms not available for plants, with a consequent reduction in the efficiency of nitrogen fertilizers (Olk et al., 2009). In our study, no deficiency symptoms on leaf lettuce was noted. Nevertheless, for the soil of seven farms, a significant increase in plant biomass was observed

with MF treatment compared to US, suggesting that soil sickness in monoculture of baby leaf can be partially associated to a non-optimal level of soil mineral nutrients.

An increase in plant biomass was also observed when the soils were sterilized, i.e, SF > SF+AC > AS > SF+AC+MF. As previously reported, soil disinfection affects both chemical and biological soil properties. Tanaka et al (2003) found an increase in the ammonium and organic nitrogen levels after the soil disinfection (both with steam sterilization and fumigation), probably due to the decomposition of microbial debris by surviving microbes. Disinfection treatments significantly affected also the presence of soil fungi. As a result, plants grew better in disinfected than untreated soil (Tanaka et al., 2003). Several authors found that, in monoculture system, the increase of soilborne pathogens or shifting in microbial community composition are the main cause of soil sickness (Li et al., 2015; Xiong et al., 2015; Yang et al., 2012; Zhou et al., 2014). In intensive agriculture, alteration of soil biota is due to several agricultural practices, including monoculture, low input of organic material, heavy application of chemical fertilizers and agrochemicals (Bonanomi et al., 2016; Sugiyama et al., 2010). In particular, the presence of the same plant species causes a reduction of microbial biodiversity on one hand, and an increase in specific pathogens on the other, with negative effects on some soil processes like humification, natural soil suppressiveness and degradation of toxic compounds (Zucconi, 2003).

In monoculture or short rotation, the growth of plants can be inhibited by the presence of toxic compounds released by plants or microorganisms into the environment (John et al., 2010; Singh et al., 1999). Asao et al. (2004) found that vanillic acid present in root exudates of lettuce have a phytotoxic effect on this plant, resulting in a reduction of the biomass of ~39% as compared with control. In our study, AC treatment was performed to assess if toxic mechanism was responsible for soil sickness in baby leaf cultivation. However, compared with US, a statistically significant increase in plant biomass was observed only for the soil of one farm, suggesting that autotoxicity was not the main mechanism involved in soil sickness of baby leaf cultivation.

5. Conclusions

Soil sickness is complex phenomenon in which a multitude of factors are involved, and the inconsistency of its expression cause difficult identification of the underlying causes. Here, an explorative study about soil sickness of baby leaf cultivation was conducted to evaluate the spread of this problem in a cultivated area of Southern Italy, and to identify the main factors

involved. Our results revealed that soil sickness of baby leaf is common in the Piana del Sele for most of sampled farms. Soil treatments significantly improved seed germination and/or plant growth. However, a heterogeneous response was observed among the applied treatments. Generally, AS treatment was the most effective in improving seed germination rate, whereas MF and SF treatments positively affected plant growth. These results indicate that there are not clear main factors involved in soil sickness. However, the positive effects of soil sterilization indicate that soil biota could have a direct effect on plant, or indirect by affecting some soil properties. Further studies are required to elucidate the mechanisms and the role of several biotic and abiotic factors involved in soil sickness.

6. References

Asao, T., Kitazawa, H., Ban, T., Pramanik, M.H.R., Matsui, Y., Hosoki, T., 2004. Search of autotoxic substances in some leaf vegetables. Journal Of The Japanese Society For Horticultural Science **73**: 247–249.

Bekunda, M., 1999. Farmers ' responses to soil fertility decline in banana-based cropping systems of Uganda. Managing Africa's Soils.

Bewley, J.D., Black, M., 1994. Seeds, physyology of development and germination.

Bonanomi, G., D'Ascoli, R., Antignani, V., Capodilupo, M., Cozzolino, L., Marzaioli, R., Puopolo, G., Rutigliano, F.A., Scelza, R., Scotti, R., Rao, M.A., Zoina, A., 2011. Assessing soil quality under intensive cultivation and tree orchards in Southern Italy. Applied Soil Ecology **47**: 184–194.

Bonanomi, G., De Filippis, F., Cesarano, G., La Storia, A., Ercolini, D., Scala, F., 2016. Organic farming induces changes in soil microbiota that affect agro-ecosystem functions. Soil Biology and Biochemistry **103**.

Chen, Y., Gamliel, A., Stapleton, J.J., Aviad, T., 1991. Chemical, physical, and microbial changes related to plant growth in disinfested soils, in: Soil Solarization. CRC Press Boca Raton, FL, pp. 103–129.

Dyer, E.W., 1995. Exploiting Weed Seed Dormancy and Germination Requirements through Agronomic Practices. Weed Science **43**: 498–503.

Engels, C., Kirkby, E., White, P., 2011. Mineral Nutrition, Yield and Source-Sink Relationships, Marschner's Mineral Nutrition of Higher Plants: Third Edition. Elsevier Ltd.

Hartemink, A.E., Poloma, S., Maino, M., Powell, K.S., Egenae, J., O'Sullivan, J.N., 2000. Yield decline of sweet potato in the humid lowlands of Papua New Guinea. Agriculture, Ecosystems and Environment **79**: 259–269.

He, C.N., Gao, W.W., Yang, J.X., Bi, W., Zhang, X.S., Zhao, Y.J., 2009. Identification of autotoxic compounds from fibrous roots of Panax quinquefolium L. Plant and Soil **318**: 63–72.

Howeler, R.H., Cadavid, L.F., 1990. Short-and long-term fertility trials in Colombia to determine the nutrient requirements of cassava. Fertilizer Research **26**: 61–80.

Huang, L.F., Song, L.X., Xia, X.J., Mao, W.H., Shi, K., Zhou, Y.H., Yu, J.Q., 2013. Plant-Soil Feedbacks and Soil Sickness: From Mechanisms to Application in Agriculture. Journal of Chemical Ecology **39**: 232–242.

John, J., Shirmila, J., Sarada, S., Anu, S., 2010. Role of Allelopathy in vegetables crops production. Allelopathy Journal **25**: 275–312.

Ju, X.T., Kou, C.L., Christie, P., Dou, Z.X., Zhang, F.S., 2007. Changes in the soil environment from excessive application of fertilizers and manures to two contrasting intensive cropping systems on the North China Plain. Environmental Pollution **145**: 497–506.

Kulmatiski, A., Beard, K.H., Stevens, J.R., Cobbold, S.M., 2008. Plant-soil feedbacks: A meta-analytical review. Ecology Letters **11**: 980–992.

Li, J.G., Ren, G. Di, Jia, Z.J., Dong, Y.H., 2014. Composition and activity of rhizosphere microbial communities associated with healthy and diseased greenhouse tomatoes. Plant and Soil **380**: 337–347.

Li, X., Zhang, Y., Ding, C., Jia, Z., He, Z., Zhang, T., Wang, X., 2015. Declined soil suppressiveness to Fusarium oxysporum by rhizosphere microflora of cotton in soil sickness. Biology and Fertility of Soils **51**: 935–946.

Marschner, P., 2012. Marschner's Mineral Nutrition of Higher Plants, Marschner's Mineral Nutrition of Higher Plants.

Martínez, M.A., Martínez, M.C., Bielza, P., Tello, J., Lacasa, A., 2011. Effect of biofumigation with manure amendments and repeated biosolarization on Fusarium densities in pepper crops. Journal of Industrial Microbiology and Biotechnology **38**: 3–11.

Mazzoleni, S., Bonanomi, G., Incerti, G., Chiusano, M.L., Termolino, P., Mingo, A., Senatore, M., Giannino, F., Carteni, F., Rietkerk, M., Lanzotti, V., 2015. Inhibitory and toxic effects of extracellular self-DNA in litter: A mechanism for negative plant-soil feedbacks? New Phytologist **205**: 1195–1210.

Minuto, A., Minuto, G., Migheli, Q., Gullino, M.L., Garibaldi, A., 2002. The "stanchezza" (soil sickness) of sweet basil. Phytopathologia Mediterranea **41**: 85–91.

Nayyar, A., Hamel, C., Lafond, G., Gossen, B.D., Hanson, K., Germida, J., 2009. Soil microbial quality associated with yield reduction in continuous-pea. Applied Soil Ecology **43**: 115–121.

Olk, D.C., Anders, M.M., Filley, T.R., Isbell, C., 2009. Crop Nitrogen Uptake and Soil Phenols Accumulation under Continuous Rice Cropping in Arkansas. Soil Science Society of America Journal **73**: 952.

Pankhurst, C.E., Blair, B.L., Magarey, R.C., Stirling, G.R., Garside, A.L., 2005. Effects of biocides and rotation breaks on soil organisms associated with the poor early growth of sugarcane in continuous monoculture. Plant and Soil **268**: 255–269.

Scarascia-Mugnozza, G., Sica, C., Russo, G., 2012. Plastic Materials in European Agriculture: Actual Use and Perspectives. Journal of Agricultural Engineering **42**: 15–28.

Singh, H.P., Batish, D.R., Kohli, R.K., 1999. Autotoxicity: concept, organisms, and ecological significance. Critical Reviews in Plant Sciences **18**: 757–772.

Sugiyama, A., Vivanco, J.M., Jayanty, S.S., Manter, D.K., 2010. Pyrosequencing Assessment of Soil Microbial Communities in Organic and Conventional Potato Farms. Plant Disease **94**: 1329–1335.

Tagliavini, M., Marangoni, B., 1992. Growth of peach as affected by decomposition of own root residues in soil. Plant and Soil **145**: 253–260.

Tanaka, S., Kobayashi, T., Iwasaki, K., Yamane, S., Maeda, K., Sakurai, K., 2003. Properties and metabolic diversity of microbial communities in soils treated with steam sterilization compared with methyl bromide and chloropicrin fumigations. Soil Science and Plant Nutrition **49**: 603–610.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature **418**: 671–7.

Wang, M., Wu, C., Cheng, Z., Meng, H., Zhang, M., Zhang, H., 2014. Soil Chemical Property Changes in Eggplant / Garlic Relay Intercropping Systems under Continuous Cropping **9**.

Xiong, W., Li, Z., Liu, H., Xue, C., Zhang, R., Wu, H., 2015. The Effect of Long-Term Continuous Cropping of Black Pepper on Soil Bacterial Communities as Determined by 454 Pyrosequencing 1–13.

Yamamoto, T., Ultra, V.U., Tanaka, S., Sakurai, K., Iwasaki, K., 2008. Effects of methyl bromide fumigation, chloropicrin fumigation and steam sterilization on soil nitrogen dynamics and microbial properties in a pot culture experiment. Soil Science and Plant Nutrition **54**: 886–894.

Yang, J. in, Ruegger, P.M., McKenry, M. V., Becker, J.O., Borneman, J., 2012. Correlations between Root-Associated Microorganisms and Peach Replant Disease Symptoms in a California Soil. PLoS ONE **7**.

Yu, J.Q., Lee, K.S., Matsui, Y., 1993. Effect of the addition of activated charcoal to the nutrient solution on the growth of tomato in hydroponic culture. Soil Science and Plant Nutrition **39**: 13–22.

Zeng, R.S., Mallik, A.U., Luo, S.M., 2008. Allelopathy in Sustainable Agriculture and Forestry.

Zhou, X., Gao, D., Liu, J., Qiao, P., Zhou, X., Lu, H., Wu, X., Liu, D., Jin, X., Wu, F., 2014. Changes in rhizosphere soil microbial communities in a continuously monocropped cucumber (Cucumis sativus L.) system. European Journal of Soil Biology **60**: 1–8.

Zhou, X., Wu, F., 2015. Changes in soil chemical characters and enzyme activities during continuous monocropping of cucumber (Cucumis sativus). Pakistan Journal of Botany **47**: 691–697.

Zucconi, F., 2003. Declino del suolo e stanchezza del terreno. Pitagora.

Figures and tables

Fig. 1. Overview of the study area and production system including: a) location of the study area in Southern Italy; b) top view of the Piana del Sele and distribution (yellow symbols) of the selected farms; c) plastic tunnels used for vegetables cultivation; d) detail of baby leaf cultivation under plastic tunnel; e-f) symptom of soil sickness in baby leaf lettuce and rocket.

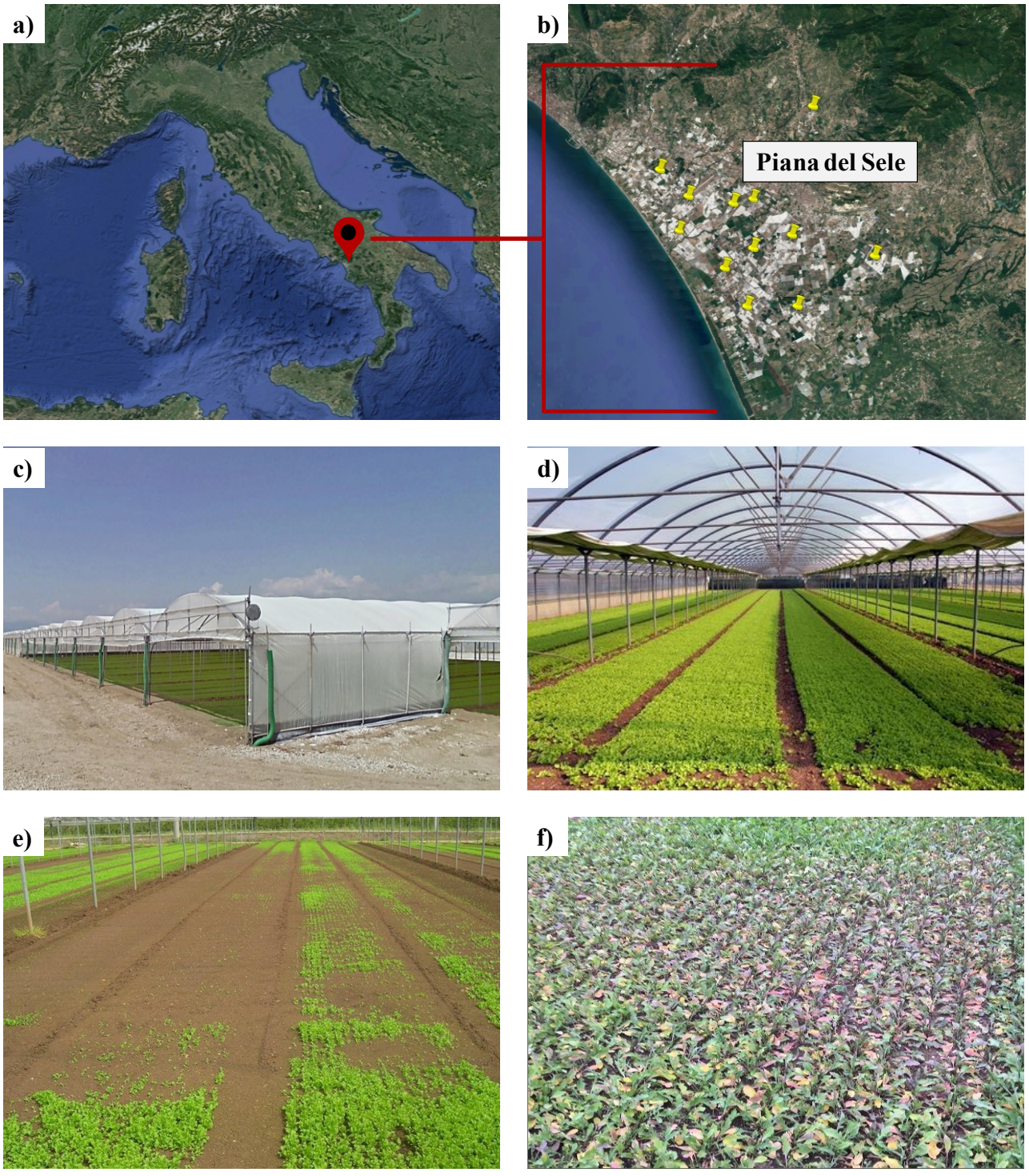


Fig. 2. Pooled results of different soil treatments. (a) Percentage of germination ten days after sowing. (b) Shoot biomass after 40 days of growth.

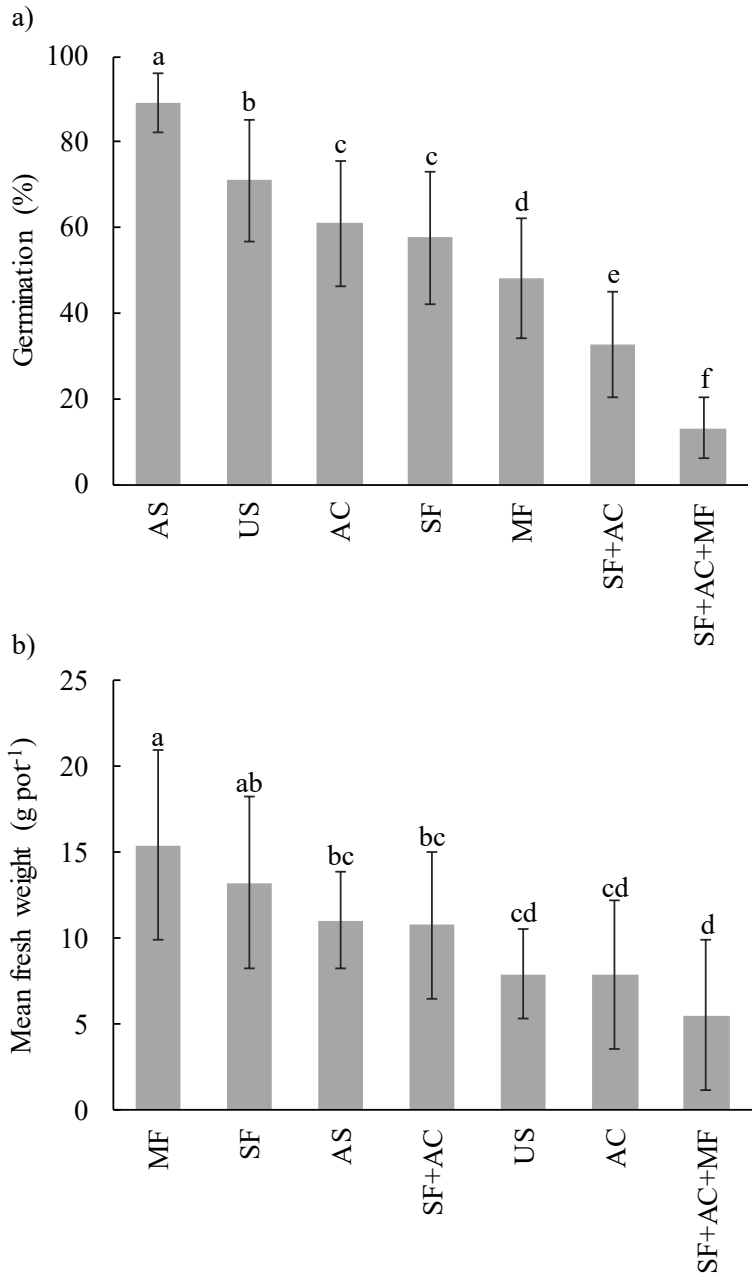
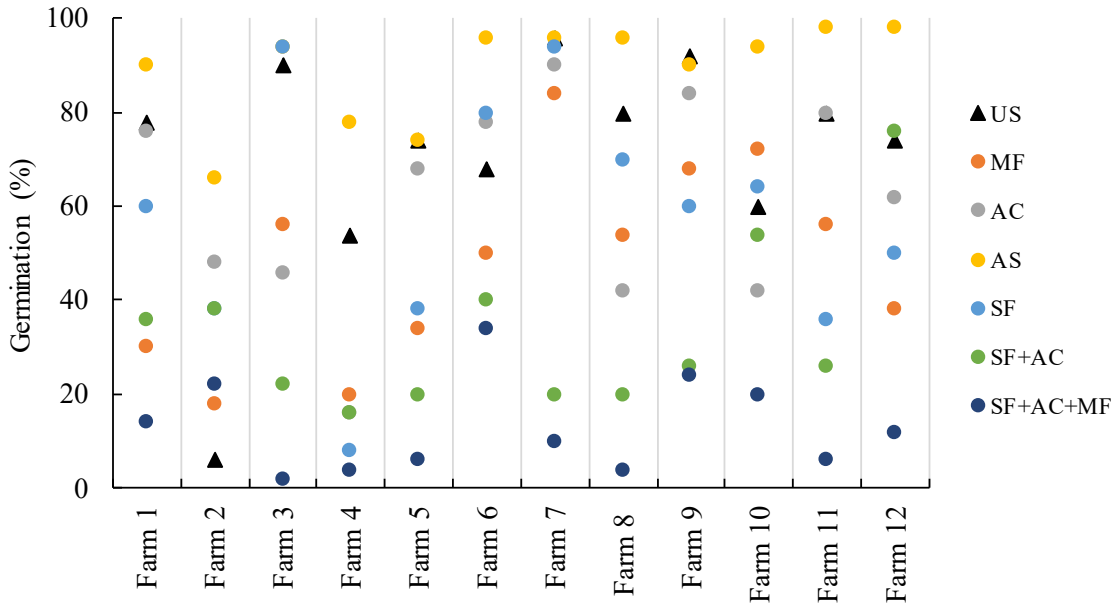


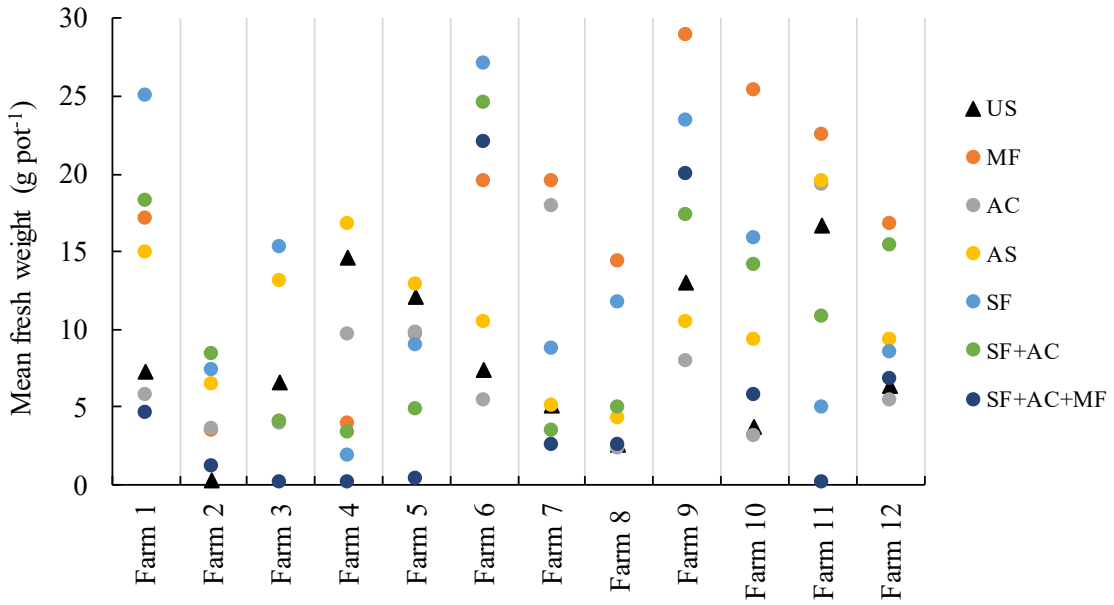
Fig. 3. Effect of soil treatments on seed germination of lettuce sown in soil from twelve different farms. Mean values and significant differences are reported in the table below.



	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7	Farm 8	Farm 9	Farm 10	Farm 11	Farm 12
US	78 ab	6 d	90 a	54 b	74 a	68 bc	96 a	80 ab	92 a	60 b	80 a	74 ab
MF	30 c	18 cd	56 b	20 c	34 c	50 cd	84 a	54 cd	68 ab	72 ab	56 b	38 c
AC	76 ab	**48 ab**	46 b	16 c	68 ab	78 ab	90 a	42 d	84 ab	42 bc	80 a	62 bc
AS	90 a	**66 a**	94 a	**78 a**	74 a	**96 a**	96 a	96 a	90 a	**94 a**	98 a	98 a
SF	60 b	**38 bc**	94 a	8 c	38 bc	80 ab	94 a	70 bc	60 b	64 ab	36 bc	50 bc
SF+AC	36 c	**38 bc**	22 c	16 c	20 c	40 d	20 b	20 e	26 c	54 bc	26 cd	76 ab
SF+AC+MF	14 c	22 cd	2 c	4 c	6 c	34 d	10 b	4 e	24 c	20 c	6 d	12 d

Value represent the percentage of seed germination (mean value) of five replicates.
Different letter within each column indicate significant differences (Duncan test, $p < 0.05$).
Bold values within each column express a significant positive difference as compared to the respective control (US)

Fig. 4. Effect of soil treatments on shoot biomass of lettuce growth in soil from twelve different farms. Mean values and significant differences are reported in the table below.



	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Farm 7	Farm 8	Farm 9	Farm 10	Farm 11	Farm 12
US	7.2 cd	0.2 b	6.5 b	14.6 a	12.1 a	7.3 c	5.1 bc	2.5 b	13.0 cd	3.7 c	16.6 ab	6.3 c
MF	**17.0 ab**	3.4 ab	4.0 b	3.9 c	9.7 a	**19.4 b**	**19.4 a**	**14.3 a**	**28.9 a**	**25.3 a**	22.5 a	**16.7 a**
AC	5.8 cd	3.5 ab	3.9 b	9.7 b	9.7 a	5.4 c	**17.9 a**	2.3 b	7.9 d	3.1 c	19.2 ab	5.4 c
AS	14.9 bc	**6.4 a**	**13.1 a**	16.7 a	12.8 a	10.5 c	5.1 bc	4.3 b	10.5 cd	9.3 bc	19.5 ab	9.3 bc
SF	**24.9 a**	**7.4 a**	**15.3 a**	1.9 c	9.0 a	**27.1 a**	8.7 abc	**11.7 a**	**23.4 ab**	**15.9 b**	4.9 cd	8.5 bc
SF+AC	**18.2 ab**	**8.4 a**	4.0 b	3.3 c	4.8 ab	**24.5 ab**	3.4 c	4.9 b	17.3 bcd	**14.1 b**	10.8 bc	**15.4 ab**
SF+AC+MF	4.5 d	1.1 b	0.2 c	0.2 c	0.3 b	**22.0 ab**	2.6 c	2.5 b	19.9 abc	5.8 c	0.1 d	6.8 c

Value represent the mean fresh weight (g pot^{-1}) of five replicates.
Different letter within each column indicate significant differences (Duncan test, $p < 0.05$).
Bold values within each column express a significant positive difference as compared to the respective control (US)

Table 1. Summary of 7 soil treatments and the related mechanisms.

Code	Treatment	Related mechanism
US	Untreated	
MF	Mineral fertilizer	Nutritional
AC	Activated carbon	Toxic
AS	Autoclave sterilization	Parasitic
SF	Soil fumigation	Parasitic
SF+AC	Soil fumigation + Activated carbon	Parasitic + toxic
SF+AC+MF	Soil fumigation + Activated carbon + Mineral fertilizer	Parasitic + toxic + nutritional

Chapter 3

ORGANIC AMENDMENT TYPE AND APPLICATION FREQUENCY AFFECT CROP YIELDS, SOIL FERTILITY AND MICROBIOME COMPOSITION

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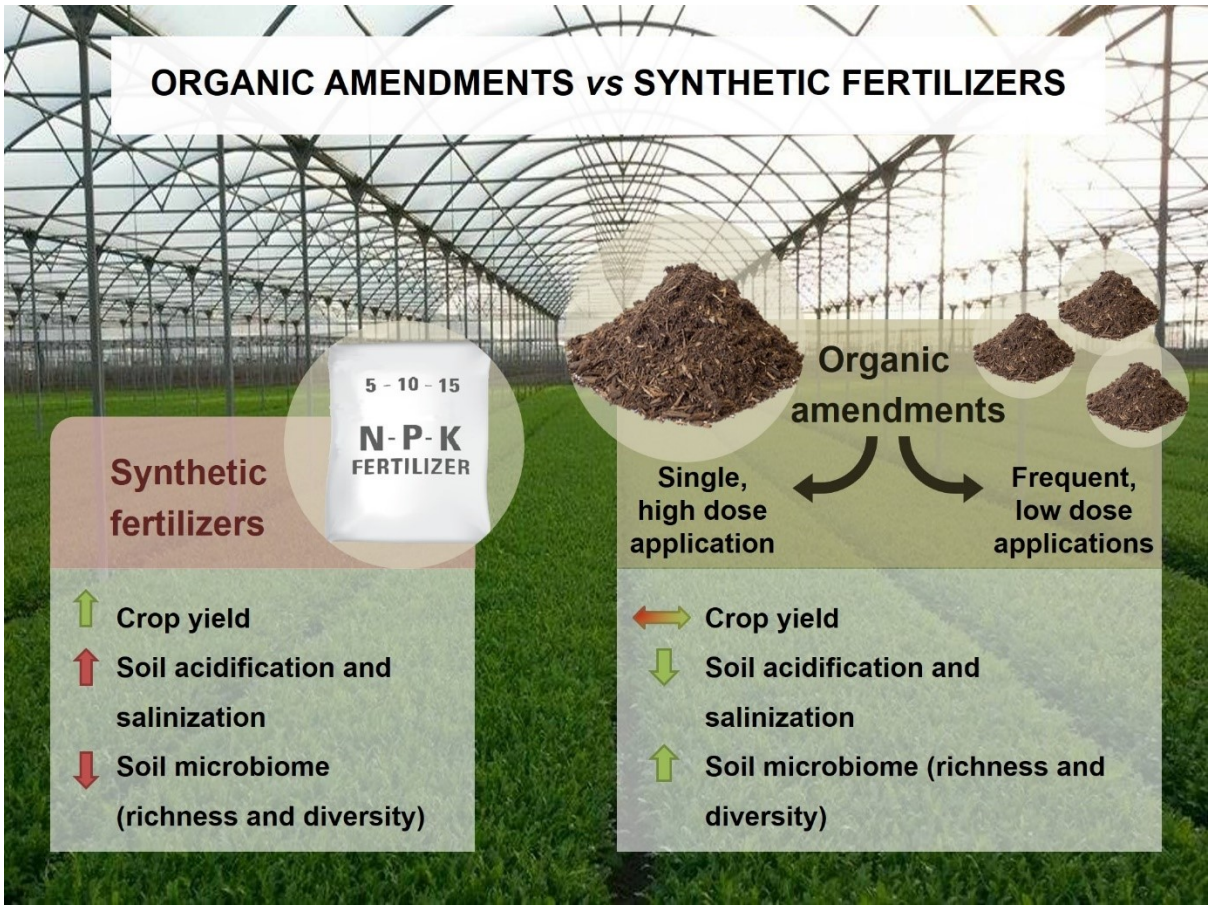

- A 1-years mesocosm experiment compared organic amendment and synthetic fertilizers
- Crop yield, soil fertility and microbiome was analysed
- Synthetic fertilizers had a higher crop production, but negatively affected soil fertility
- 454 pyrosequencing revealed difference between synthetic fertilizers and organic amendments
- Organic amendments increase microbiome diversity and improved their functionality

Abstract

Vegetable cultivation under plastic tunnels provides high-quality crop yields but negatively affects soil fertility, especially when conventional agricultural system (e.g., use of synthetic mineral fertilizer and agrochemicals) is adopted. In this regard, the use of organic amendments has been proposed as a reliable and effective approach for soil fertility recovery. The aim of this study was to assess the effects of different organic amendments and application frequencies on crop yield, soil fertility and soil microbial communities. A 1-year long mesocosm experiment was performed by conditioning a soil with different organic amendment types and frequency of application, compared with the ordinary soil management based on mineral fertilizers and fumigation. Soil fertility was assessed by determining physical and chemical soil parameters, whereas microbial community functioning and structure were assessed by high-throughput sequencing of bacterial and eukaryotic rRNA gene markers and BIOLOG EcoPlates™. Compared to the organic amendment, the use of synthetic fertilizer had a higher crop production but negatively affected pH, soil organic carbon content and soil aggregation. Diversity and richness of bacteria and eukaryotic were lower in the synthetic than in the organic amendments. The addition of organic amendments promoted the growth of Acidobacteria and Gemmatimonadetes bacteria. On the contrary, members of Actinobacteria and Proteobacteria were more abundant in the soil treated with synthetic fertilizer. This study increases our current knowledge on the effect of the synthetic and organic amendment applications on crop yield, soil fertility and soil microbial community functionality.

Key Words: Organic carbon, disease suppression, microbial functional diversity, high-troughput pyrosequencing, soil “sickness”.

1. Introduction

The use of organic amendments such as animal and green manure (Himmelstein et al., 2014; Tejada et al., 2009), organic wastes (Croteau and Zibilske, 1998; Torres et al., 2015), composts (Bastida et al., 2015; Noble and Coventry, 2005), and biochar (Jones et al., 2012; Lehmann et al., 2011), has been proposed as a reliable and effective approach for soil fertility recovery (Diacono and Montemurro, 2010; Haynes and Naidu, 1998). Different mechanisms have been proposed to explain the positive effects of organic amendments on soil fertility and health, including increase of microbial activity (Melero et al., 2006), enhanced soil structure (Bronick and Lal, 2005), release of mineral nutrients during organic matter decomposition (Berry et al., 2002), and induction of disease suppression towards soilborne pathogens (Bulluck et al., 2002). Unfortunately, the use of organic amendments has also significant drawback effects that limit their applicability in agro-ecosystems. For instance, the suppressive capability of organic amendments is often inconsistent and many studies report an increase of disease incidence after organic matter application (reviewed in Bonanomi et al., 2007). To eliminate these inconsistencies and successfully apply organic amendments it is necessary to understand the factors that affect the impact of different organic amendments types on soil microbiome and, then, on soil functioning.

A large variety of organic matter types including crop residues, composts, peats, organic wastes from agro-industries, and biochar are widely used as soil amendments. Most of the published studies assessed the immediate biological and agronomic effects of organic amendments by adding them only once at the start of the experiment (e.g. Bastida et al., 2008; Ferreras et al., 2006), or by repeating the treatment usually once a year in long-term field trials (Diacono and Montemurro, 2010; García-Gil et al., 2000; Marschner et al., 2003; Ros et al., 2006; Steiner et al., 2007). This experimental approach mirrors the ordinary agricultural practices where organic amendments are usually added to soil once or twice a year. In agro-ecosystems, however, organic carbon inputs including rhizodeposition and crop residues follow complex dynamics in relation to soil management, which varies with crop successions, type and frequency of organic amendment application. In this regard, only few studies addressed the link between frequency of soil amendments and soil functioning. The effect of organic C application frequency has been studied in relation to soil basal respiration (Nett et al., 2012), enzymatic activities (Stark et al., 2008), soil fungistasis (Bonanomi et al., 2016b), and nitrogen (N) mineralization (Duong et al., 2009; Mallory and Griffin, 2007). Previous studies demonstrated that soils subjected to repeated organic amendment applications,

compared with single applications, have a higher and more active microbial biomass (Kandeler et al., 1999) and enhanced enzymatic activities (Dick et al., 1988). These considerations about basic soil processes and history of organic inputs suggest that conditioning soil with repeated organic matter inputs can positively affect soil fertility and health by stimulating the activity of the resident microbial community. However, most of these studies were based on short-term, laboratory incubation experiments that used model organic compounds as C sources (e.g. glucose, glycine etc). As a consequence, it is still difficult to translate the available knowledge about the effect of repeated C applications into effective practical applications in real agricultural systems.

Cultivation under plastic tunnels is a growing agricultural sector with about 2 million ha in the world and more than 190,000 ha in the Mediterranean Basin (Scarascia-Mugnozza et al., 2012). This cultivation system provides several advantages due to the improvement of microclimatic conditions, relatively low investment costs and, thanks to high-quality crop yields, elevate net income for farmers (Belasco et al., 2013). However, this type of cultivation negatively affects soil properties because it drastically modifies water, carbon, and nutrient cycles. The almost complete rainfall restriction and the consequent requirement of localized irrigation to support crop water demand increases soil salinity, while the widespread use of synthetic mineral fertilizers induces soil acidification (Ju et al., 2007). Also the use of fumigants has a negative effect on soil fertility and crop health, affecting the soil microbial composition and the development of pesticide resistances (Tilman et al., 2002). In addition, the systematic elimination of crop residues to limit plant diseases, the optimal temperature and water content that promote mineralization of organic matter induce a reduction of soil organic carbon content, with a negative feedback on soil microbial communities. A crucial step for a sustainable management of soil fertility is to identify organic amendments with such a chemistry that effectively improves soil microbial activity, enhance soil fertility and provide mineral nutrients by mineralization (Bonanomi et al., 2014).

In this work, we experimentally explored the possibility of an effective enhancement of soil fertility and crop yield by using organic substrates including biochar, animal manure and crop residues applied with different frequencies. For this purpose, a 1-year long mesocosm experiment was performed by conditioning a soil with 11 treatments of organic amendments differing for the types and frequency of application. In detail, we hypothesized that frequent addition of organic amendments, thanks to the continuous supply of easily decomposable organic compounds, enhance microbial activity and diversity and promote soil fertility and plant productivity.

The impact of different soil managements, including ordinary farming approaches based on mineral fertilizers and soil fumigation, was assessed on crop yield of rocket (*Eruca sativa*), soil chemistry, and microbial community functioning and structure by high-throughput sequencing of bacterial and eukaryotic rRNA gene markers and BIOLOG EcoPlates™. The main objectives of our study were to assess:

(i) the impact of organic amendment types and frequency on crop productivity and soil chemistry;

(ii) the differences in microbiota composition between soil managed with ordinary farming practices and soil treated with different organic amendments;

(iii) the relationships between microbiota composition, soil properties and crop yield.

2. Material and methods

2.1. Organic amendments and soil collection

Four types of organic substrates were selected, having different C/N ratios and N content (values are average ± standard deviations) as follows: i) alfalfa straw (*Medicago sativa*) (N content = 3.93 ± 2.16%; C/N ratio = 11.43 ± 2.98); ii) glucose (N content = 0.00; C/N ratio = ∞); iii) compost manure (N content = 3.13 ± 0.64%; C/N ratio = 13.09 ± 1.16); iv) wood biochar (N content = 0.51 ± 0.11%; C/N ratio = 149.61 ± 7.26). Alfalfa straw was sampled from agricultural fields, air dried for 20 days until reaching constant weight, finely powdered using a ball mill and stored afterwards at room temperature. Compost manure was obtained by mixing cattle faeces and straw in forced-aeration pile. At the end of the process, compost manure was collected from cattle farm, air dried at room temperature until a constant weight was reached, finely powdered and then stored at room temperature. Soil was collected from a farm located in a productive area of about 5,000 ha cultivated under greenhouses located in the Salerno area (Southern Italy; 40°33'13"N, 14°57'22"E). Low-technology, unheated polyethylene-covered greenhouses (height ~4 m) are the main crop protection structures used in this area. The study site had a Mediterranean climate with a mean annual temperature of 15.9°C and mean monthly temperatures ranging from 23.6°C in August to 9.0°C in January. The climate has a mean annual rainfall of 988 mm with a relatively dry summer (84 mm). The farm adopted an intensive farming system for ~10 years based on cultivation under the plastic tunnel, intensive tillage with an average of 6 plowing treatments every year including rototilling, spading and harrowing (Bonanomi et al., 2011). In spring 2013 about 1,000 kg of soil were collected from the first 20 cm layer. The soil had a silt loam texture (22.1% clay, 56.6% silt, 21.3% sand),

with a pH of 7.74 and an electrical conductivity (EC) of 0.32 dS m^{-1}; it contained 15.4 g kg^{-1} of organic carbon and 1.6 g kg^{-1} of total N, with a C/N ratio of 9.6 (for the other soil properties see Supplemental Table S1).

2.2. Mesocosm experiment

The experiment compared the ordinary cultivation method, based on mineral fertilizers and fumigation with Metham-Na, with the use of different organic amendment types and frequency of application. Organic amendments were combined considering the complementary properties of the organic substrate: e.g. glucose provides short term labile C for microbes, *Medicago sativa* hay and compost manure have more recalcitrant C and are source of organic N, and biochar provides a safe site for microbial development and promotes soil physical properties. In detail, the experiment had a total randomized design, including 11 soil treatments (thereafter indicated as ST) with 3 replications each for a total of 33 experimental units (Table 1, Fig. S1). The 11 STs were so composed: ST 1 - untreated soil (control); ST 2 – soil treated with synthetic fertilizers; ST 3 - soil fumigated by Metham-Na and treated with synthetic fertilizers; ST 4 – soil with a high rate, single application of compost manure at the start of the experiment; ST 5 - soil with a high rate, single application of compost manure plus wood biochar at the start of the experiment; ST 6 – soil with a high rate, single application of glucose and alfalfa straw at the start of the experiment; ST 7 - soil with a high rate, single application of glucose and alfalfa straw plus wood biochar at the start the experiment; ST 8 - soil with low application rates of compost manure added weekly during crop growth; ST 9 - soil with low application rates of compost manure added weekly during crop growth plus wood biochar at the start of the experiment; ST 10 - soil with low application rates of glucose and alfalfa straw added weekly during the whole experiment; ST 11 - soil with low application rates of glucose and alfalfa straw added weekly during the whole experiment plus wood biochar at the start of incubation (Table 1). Biochar (size <1 cm) was incorporated into the soil once at the start of the experiment at the dose of 30 t ha^{-1}. For single applications, powdered organic materials were incorporated into the soil at the doses of 15 t ha^{-1} for compost manure, 13 t ha^{-1} for alfalfa, 7 t ha^{-1} for glucose. Unlike single dose, weekly applications were performed by shedding liquid extract of organic amendments on the soil surface. Considering the high crop density (i.e., 600 mg/m^2 of seeds), a different application method was used to avoid staining of the leaves with organic amendments. Liquid extract was prepared by mixing organic material and water using a 1:2 ratio, vigorously shaking it for 5 hours and filtering the mixture. Then, liquid extract was

distributed on soil surface, below crop canopy. The amount of organic material used for weekly applications corresponded to 0.43 t ha^{-1} for compost manure, 0.37 t ha^{-1} for alfalfa, 0.2 t ha^{-1} for glucose.

The collected soil was sieved in the laboratory (mesh size <2 mm) and mesocosms consisting in 32 L plastic tray were filled with 35 kg of soil and brought to 85% of water field capacity. Thereafter, the mesocosms were incubated in a greenhouse equipped with automatic control of temperature. The temperature was kept at 24 ± 4°C day and 18 ± 4°C night in spring and summer and 18 ± 4°C day and 12 ± 4°C in fall and winter. During the experiment, every three days the mesocosms were irrigated to 85% of field capacity by a sprinkler irrigation system. The experiment lasted for 360 days.

2.3. Crop cultivation and yield

Six consecutive cycles of rocket (*Eruca sativa*) cultivation were made during the mesocosm experiment. This method mirrors the ordinary cultivation method used by farmers in Southern Italy. Briefly, rocket was sown by hand spreading 600 mg m^{-2} of seeds and covered with a thin layer (~3 mm) of soil. The length of the cycles ranged from about ~35 to ~50 days in summer and winter, respectively. Crop yields were recorded at the end of each cycle quantifying the amount of commercial production by cutting the plant at ground level in all mesocosms. The material was air-dried in a dehydrator until reaching a constant weight that was thereafter recorded.

2.4. Soil properties

At the end of the experiment, i.e. after 360 days of cultivation, soil samples were collected from each mesocosm and transferred to the laboratory. Soil chemical properties were determined by standard methods (Sparks, 1996) on soil air dried at +25 °C until constant weight was reached and sieved through 2 mm mesh. Electrical conductivity (EC) and pH were measured in 1: 5 and 1: 2.5 soil: water suspensions, respectively. Organic C content was assayed by chromic acid titration method (Walkley and Black, 1934); ammonium ($N\text{-}NH_4^+$) and nitrate ($N\text{-}NO_3^-$) contents were assayed by using ion-selective electrodes specific for ammonium and nitrate.

Water stability of soil aggregates (WSA) was assessed according to the method of Kemper and Rosenau (1986). Twenty grams of air dried soil were sieved through 4.75 mm mesh and put in the highest of a sequence of three sieves of 1.00, 0.50, and 0.25 mm mesh size. The soil

was pre-soaked in distilled water for 30 min, then the nest of sieves and its contents were oscillated vertically in water 20 times using a 4 cm amplitude at the rate of one oscillation per sec. After wet-sieving, the resistant soil materials on each sieve, including unstable aggregates (< 0.25 mm), were recovered, dried in the oven at 50 ◦C for 48 h and weighed. Aggregates stability were expressed as a mean-weight-diameter (MWD) value which is the sum of the mass fraction of soil remaining on each sieve after sieving, multiplied by the mean diameter of the adjacent meshes (Spaccini et al., 2004).

2.5. Soil microbiological analyses

Microbiological analysis were carried within three days of sampling on fresh soils stored at +4 °C. The composition and diversity of soil microbial community was analyzed by high-throughput sequencing, whereas the functionality of the soil microbial community was evaluated by BIOLOG EcoPlates™.

Since one of the objectives of this study was to evaluate the differences in microbiota composition between soil managed with ordinary farming practices and soil treated with different organic amendments, DNA was extracted in triplicate for each soil treatment, but the successive amplification was done by pooling the DNA of the three replicas. Total DNA extraction from soil samples (0.25 g) was carried out by using the PowerSoil DNA Isolation kit (Mo Bio Laboratories Inc., Carlsbad, CA). The bacterial and eukaryotic diversity were studied through pyrosequencing of the V1-V3 regions of the 16S rRNA gene (about 520 bp) and a portion of the 18S rRNA gene (about 436 bp), respectively, by using primers and PCR conditions previously reported (Bonanomi et al., 2016a; Ercolini et al., 2012). PCR products were purified with the Agencourt AMPure kit (Beckman Coulter, Milan, IT) and quantified using a Plate Reader AF2200 (Eppendorf, Milan, IT). Equimolar pools were obtained prior to further processing and pyrosequenced on a GS Junior platform (454 Life Sciences, Roche Diagnostics, IT), according to the manufacturer's instructions.

Community-level physiological profile (CLPP) of microbial populations was performed by the BIOLOG EcoPlates™ (BLG) method based on carbon substrate utilization. BLG consists of 96 wells containing 31 different carbon sources and a blank in triplicate. When the carbon source is utilized, the tetrazolium violet dye is reduced by developing a purple colour. The assay was performed as previously described by Bartelt-Ryser et al. (2005). Briefly, 1 g of sieved soil (mesh 2 mm) was shaken for 30 min in 10 ml of distilled water and then allowed to settle for 10 min. Then, 120 µl of the supernatant were diluted 100-fold in distilled water,

mixed, and finally used to inoculate the wells of the BIOLOG EcoPlates™. The plates were incubated at room temperature, and oxidation of carbon sources was measured with a spectrophotometer (Thermomax microtitre plate reader, Molecular Devices, Wokingham, UK) at 590 nm, after 24, 48, 72 and 96 h of incubation. Average well colour development (AWCD) was calculated as the sum of wells with activity per plate, divided by the 31 carbon sources.

2.6. Bioinformatics data analysis

Raw reads were filtered and analysed by using the QIIME 1.9.0 software (Caporaso et al., 2010). Reads shorter than 300 bp, with more than 1 primer mismatch and with average quality score lower than 25 were discarded. Operational taxonomic units (OTUs) were picked through a *de novo* approach and uclust method and taxonomic assignment were obtained by using the RDP classifier and the Greengenes (McDonald et al., 2012) or the Silva SSU/LSU rRNA gene database release 119 (Quast et al., 2013), for bacteria and eukarya, respectively. Chloroplast and Streptophyta contamination, as well as singletons, were removed and the relative abundance of other taxa was recalculated. In order to avoid biases due to the different sequencing depth, OTU tables were rarefied to the lowest number of sequences per sample (3,048 and 3,696 for bacteria and eukarya, respectively). Alpha-diversity analysis (observed OTU richness, Chao1 and Shannon indices) was carried out in QIIME on rarefied OTU tables. Rarefied OTU tables were imported in R environment for statistical analyses and plotting (http://www.r-project.org). Finally, PCA plot of soil samples was performed at the genus level for bacteria and eukarya by using XLSTAT software.

2.7. Statistical analysis

One-way ANOVA was used to analyse the effect of ST on crop yield and soil chemical and microbiological parameters. The relationship between relative changes in crop yield, soil properties and microbial community composition, including richness and diversity, was obtained for all the measured parameters. In detail, Pearson correlation coefficients were calculated and significance was evaluated at $p < 0.05$ and < 0.01. All statistical analyses were performed by STATISTICA 7 software.

PCA box-plot of soil samples was performed at the genus level for bacteria and eukarya by using STATISTICA 7 software. Hierarchical clustering of the samples was carried out by using average-linkage clustering based on the Pearson's correlation coefficient of the microbial or eukarya community abundance.

The 16S and 18S rRNA gene sequences are available at the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI), under accession number SRP092020.

3. Results

3.1. Crop yield

Crop yield (dry weight) was significantly affected by the soil treatments (ST) (Fig. 1). Compared with the ST 1, corresponding to untreated soil, the application of mineral fertilizers (ST 2) had the higher yield, especially when the soil was previously fumigated by Metham-Na (ST 3). Differently, with the application of organic material a large variability of yield was observed, in relation to the amendment type and application frequency. In particular, a high yield was observed when alfalfa and glucose were combined and applied in a single dose, independently of the presence of biochar. For the remaining STs, small differences were observed in crop yield as compared to ST 1 (Fig. 1).

3.2. Soil fertility

Soil chemical parameters as well as soil aggregation were affected by STs (Table 2). Soil nitrate ($N\text{-}NO_3^-$) concentration at the end of the experiment (i.e. after 360 days) was very high in ST 2 and ST 3 compared with the other STs (Table 2). Soil ammonium ($N\text{-}NH_4^+$) concentration and soil salinity (EC) showed a similar trend, with higher values for ST 2 and ST 3, with respect to the other treatments. On the contrary, pH was lowered to a level acid (pH = 5.38) and extremely acid (pH = 4.39) for ST 3 and ST 2 treatments, respectively, whereas a neutral pH was observed for the remaining STs (Table 2). The use of synthetic fertilizers (ST 2 and ST 3) led to a significant reduction of the organic carbon content compared with other STs. Organic amendment applications increased soil organic carbon content, especially when it was added as a single dose and in combination with biochar (Table 2). Finally, a higher stability of soil aggregates was observed for the treatments in which alfalfa and glucose were used as soil amendment (Table 2). In these cases, after wet-sieving more than 20% of macro-aggregates with diameter > 1.00 mm were stable in water, whereas the content of unstable aggregates (diameter < 0.25 mm) was less than 11% (Fig. 2).

3.3. Microbiome diversity, structure, and functionality

CLPP analysis carried out at the end of the experiment showed significant differences between organic and synthetic fertilizer treatments (Table 2). The highest AWCD value after 96 h of incubation was recorded for ST 11, and the lowest for treatments based on synthetic fertilizers (ST 2 and ST 3), with intermediate values for the untreated control (ST 1) and the other STs (Table 2). Data from BIOLOG EcoPlates™ for specific carbon source utilization pattern showed that the substrate classes of carbohydrates, amino acids, phosphate carbon and carboxylic acids were rapidly utilized by STs based on organic amendments (from ST 4 to ST 11) (Fig. S2). Noteworthy, amine compounds were rapidly degraded only in the ST 11 (Fig. S2).

Pyrosequencing of 16S and 18S rRNA genes were used to describe the functional diversity and underlying phylogenetic changes in response to different STs. The average number of sequences/sample obtained after the quality filtering was 5,148 and 5,072 with an average read length of 530 bp and 452 bp for bacteria and eukarya, respectively. Different soil treatments significantly affected diversity and richness metrics based on observed OTUs, Shannon and Chao1 indices (Fig. 3). Compared with application of organic amendments (ST 4 to ST 8), synthetic fertilizers (ST 2 and ST 3) reduced the number of observed OTUs. In addition, ST 2 and ST 3 had the lowest bacteria and eukarya richness, as shown by the Chao1 index (Fig. 3). Among the treatments based on organic amendment applications, the highest value of bacterial richness was observed in the soils that received biochar, but the opposite result was found for the eukaryotic community (Fig. 3). Finally, bacteria diversity was higher for the application of organic amendments than with the use of synthetic fertilizers, whereas a variable response depending on soil treatment was observed for eukaryotic diversity (Fig. 3).

Considering the bacterial composition at a phylum level, a total of 30 phyla were found in all the samples. Firmicutes (22.4%), Proteobacteria (20.8%), Acidobacteria (15.6%), Bacteroidetes (10.5%), Actinobacteria (9.4%), Gemmatimonadetes (7.2%) and Chloroflexi (4.1%) were the dominant phyla. However, the relative abundance varied considerably across treatments (Fig. 4a). Firmicutes prevailed when alfalfa and glucose were applied once at the beginning of the experiment (ST 6 and ST 7). Untreated soil (ST 1) had the lowest abundance of Proteobacteria, while Acidobacteria abundance was particularly high. On the contrary, lowest level of Acidobacteria was observed for the other STs, especially when the soil was previously fumigated (ST 3) (abundance < 1%). Similarly, the fumigation treatment strongly reduced the abundance of Chloroflexi and Gemmatimonadetes, whereas the highest values

were observed for ST 11 and ST 5, respectively. Finally, the highest abundance of Actinobacteria and Bacteroidetes communities was observed for ST 2 and ST 3 (Fig. 4a).

Considering the eukarya composition at class level, *Nucletmycea* was the dominant group of microorganisms across all the samples (Fig. 4b). Amoeboid organisms were most abundant in soil amended with organic matter. In particular, *Schizoplasmodiida* class was the most abundant in the soil amended with compost manure (ST 4 > ST 8 > ST 5 > ST 9), while *Gracilipodida* prevailed when alfalfa and glucose were applied in fractional doses (Fig. 4b). Soil fumigation (ST 3) promoted the growth of *Acanthocystidae* and of members of the SAR super-group, such as *Alveolata* and *Rhizaria*, compared to the other STs (Fig. 4b). A very low value in classified fungi at the genus level was observed for ST 3, while *Rhizopus* was the most abundant fungal genus with all the other treatments (Fig. S3). PCA based on bacteria and eukarya community composition at genus level clearly separated the samples according to different STs (Fig. S4). In particular, the analysis clustered the treatments into three groups including soil with application of synthetic fertilizers (ST 2 and ST 3), soil with single application of alfalfa (ST 6 and ST 7), and the remaining treatments (Fig. S4).

Hierarchical clustering of the bacteria and eukarya profiles for different treatments are reported in (Fig. S5 and Fig. S6). Bacterial profiles at family level clustered the treatments in two groups. The first cluster includes the treatments that showed a greater yield, i.e. the soils that had received alfalfa as a single dose (ST 6 and ST 7) and synthetic fertilizers (ST 2 and ST 3). The second cluster included the remaining STs grouped for type of amendment and frequency of application (Fig. S5). On the contrary, hierarchical clustering of the eukarya community at genus level showed a clear separation of the soils amended with compost manure (ST 4, ST 5, ST 8 and ST 9) from all the others (Fig. S6).

3.4. Linking crop yield, soil chemical quality and microbiota composition

EC, N-NH_4^+ and N-NO_3^- content were positively related to crop yield (Table 3). On the contrary, crop yield showed a significant negative correlation with pH and Biolog AWCD (Table 3). Soil properties such as EC and N-NO_3^- were positively related between them and negatively correlated with pH, organic carbon and AWCD. Finally, soil aggregation was positively affected by organic carbon and microbial activity (Table 3).

Crop yield positively related with *Bacteroidetes* and *Firmicutes* phylum, whereas strong negative correlation was found with *Acidobacteria*, *Chloroflexi* and *Gemmatimonadetes,* as well as with bacteria richness and diversity (i.e., Chao1 and Shannon index) (Table 4). Among

fungal community, positive correlation was found between crop yield and members of *Clavicipitaceae, Cunninghamellaceae, Spizellomycetaceae* and *Trichocomaceae*, but no significant correlation was found with fungal richness and diversity (Table S2). Concerning soil quality, *Actinobacteria* and several fungi members, including *Clavicipitaceae, Cunninghamellaceae, Tremellaceae* and *Trichocomaceae* showed a significant positive correlation with EC, N-NH_4^+ and N-NO_3^-, and negative correlation with pH and AWCD (Table 4 and Table S2). On the contrary, *Acidobacteria* negatively related with EC and N-NO_3^- (Table 4). Soil aggregation (MWD) showed significant positive correlation ($p = 0.05$) only with some fungi members, like *Stachybotrys, Piptocephalidaceae* and *Thamnidiaceae* (Table S2). Finally, significant correlation between soil properties and microbial richness and diversity was found only for bacterial communities (Table 4). In detail, Chao1 and Shannon index negatively related with EC, N-NH_4^+ and N-NO_3^-, and positively with AWCD (Table 4).

4. Discussion

The use of organic amendments has been proposed as an alternative to synthetic fertilizers for the improvement of soil fertility and quality and, consequently, the crop yield (Bonilla et al., 2012; Melero et al., 2006; Stockdale et al., 2002). In our study, the effects of different organic amendments, in terms of amendment types and frequency of application, on soil chemistry, microbial community composition and crop productivity were evaluated in comparison to those of synthetic fertilizers. At the end of a 1 year-long experiment, we found crop yields higher in soils added with synthetic fertilizers than in those treated with organic matter. Several studies reported that during the transitioning from conventional to organic agriculture, the crop yield is low in the first years and gradually increases over time (e.g. Bulluck et al., 2002; Martini et al., 2004). Berry et al., (2002) suggested that the addition of organic amendments such as cover crops, compost and animal manure slowly releases available mineral nitrogen with consequent N short-term limitations for the plants. In a long-term experiment to evaluate the effects of different soil managements on crop yield and soil microbial properties, Ros et al. (2006) have found that, compared to the application of mineral N fertilizers, the addition of composts alone resulted in a lower yield of maize, whereas a higher productivity was observed when composts plus mineral N fertilizers were applied. These results suggest that the addition of N readily available can help to overcome the N deficiency, especially during the period of high N demand by crops (Seufert et al., 2012). In our study, the

different N contents in soil treated with synthetic fertilizer and organic amendments can differently support the plant growth.

To understand the effects of heavy mineral fertilizer applications in soil, Ju et al. (2007) found that soil pH decreased exponentially with NO_3^- concentrations. Similarly, our results show a strong negative correlation between pH and soil NO_3^- concentration. The lower pH value found in the treatments that have received synthetic fertilizers is probably due to acidification processes deriving from the nitrification of N fertilizers (Hedley and Bolan, 2003). In the long term, the enhanced soil EC combined with the pH lowering may adversely affect the yield and quality of the crops. Moreover, excessive soil nitrate content can accumulate in plant tissues, especially leaf of vegetables, representing a hazard to the health of consumers (Santamaria et al., 1998). An increase in soil nitrogen concentration can also lead to a salinization effect, being the NO_3^- content significantly correlated with total soil salinity (Liang et al., 1997) and, consequently, have a negative influence on crop yield. In accordance with Xue et al. (1994), a significant correlation was found between NO_3^- concentration and soil EC. Considering soil pH, sub-optimal levels affect the nutrient solubility in soil water and therefore the magnitude of nutrients available to plants (Marschner, 1995). The content in soil organic matter influences the soil structure, since it is positively related with size and amount of water stable aggregates (Bronick and Lal, 2005). In agreement with Ferreras et al. (2006), in our study the addition of organic amendments and the resulting increase in soil organic matter largely explain the significant correlation found between soil organic carbon and soil aggregation. The higher organic matter content recorded in soils amended with single applications of powdered material as compared to repeated liquid applications could be partially related to these different forms of application.

The application of organic amendments is known to have positive effects on the soil microbial community structure, functioning and, therefore, crop yield (Bulluck et al., 2002; Haynes and Naidu, 1998; Melero et al., 2006). Marschner et al. (2003) have found that the repeated use of organic amendments, such as manure and sewage sludge, increased the microbial biomass and changed the microbial community structure compared to the mineral treatments. However, this difference was not observed when straw was added to the soil, suggesting that the quality of the amendment is responsible for different results. Soil microorganisms are involved in several processes, such as the transformation of soil organic matter, nutrient cycling, improvement in soil physical condition and fungistasis (Bronick and Lal, 2005; Diacono and Montemurro, 2010; Haynes and Naidu, 1998). In the report of Nielsen

and Winding (2002), increased microbial biomass and activity are regarded as indicators of improved soil health and, consequently, agricultural sustainability and crop yields. In our study, the characterization of microbial community through Biolog EcoPlates showed a higher degradation capability of several organic compound classes when soil was amended with organic matter compared to the use of synthetic fertilizers. In addition, the significant correlation found between the AWCD value and soil organic carbon explains the importance of organic matter for the microbial activity. Despite these results, we found that crop yield in soil amended with organic materials was lower than in soil treated with mineral fertilizers. The highest AWCD value of the organic STs can be related to the increase in number and size of soil microorganisms (Bronwyn et al., 1997), whereas the lowest crop yield is probably due to N limitation (Berry et al., 2002) as result of the transition from synthetic to organic amendments (Bulluck et al., 2002; Martini et al., 2004).

Biolog EcoPlate represents a simple and rapid method to discriminate differently treated soils (Garland and Mills, 1991), but it doesn't give any information about the diversity and richness of microbial species. Here, we found strong positive correlations between AWCD with Chao1 and Shannon indices, suggesting a positive link between functional and taxonomic microbial diversity. To investigate the effect that ordinary farming system (i.e., use of mineral fertilizers and soil fumigation) and different organic amendment strategies (i.e. amendment type and frequency of application) have on the composition of soil microbiota, we used a high-throughput sequencing approach. Although this analysis was conducted without true replicas, the results of PCA clearly separate the soil treated with organic amendments from those with synthetic fertilizers, indicating the presence of similarity within each group on one hand, and the difference between the two groups on the other hand. Several studies reported that land use, management type, plant species and soil properties such as pH, soil type, soil texture and nitrogen availability can affect microbial community structures. However, most of these studies focused their attention on the changes of bacterial communities (Chaudhry et al., 2012; Fierer et al., 2012; Li et al., 2012; Ma et al., 2016; Pershina et al., 2015; Ramirez et al., 2012), while only a few authors investigate the eukaryotes (Bonanomi et al., 2016a; Hartmann et al., 2015). Our results clarify that the use of organic amendments and synthetic fertilizers greatly affects both the bacterial and eukaryotic communities in terms of number of species and richness. In fact, the application of organic amendments increased the observed number of OTU and richness of microorganisms, compared to the soil subjected to applications of synthetic fertilizers, as reported by several authors (Chaudhry et al., 2012; Hartmann et al., 2015; Li et

al., 2012). These results can be related to the higher availability of organic C, in terms of global amount as well of chemical diversity, in treatments subject to periodic amendments. In addition, the use of biochar enhances the bacterial abundance and richness, as reported by Lehmann et al. (2011). These authors suggest that the surface and porosity of biochar provide a favourable microhabitat where bacteria may adhere and, consequently, be better protected against leaching, dissecation, grazers and competitors.

In our study, Firmicutes and Proteobacteria were the dominant bacterial phyla in all the treatments. The highest abundance of Firmicutes was observed in the treatments with the single application of alfalfa and glucose. These bacteria prosper in soil with high carbon availability (Pershina et al., 2015) and can degrade various complex organic materials (Hartmann et al., 2015). Some members of Proteobacteria, such as *Betaproteobacteria*, are considered copiotrophic, i.e. organisms with fast growing that prosper in nutrient-rich environments. High N inputs under intensive cultivation systems seems responsible for the shifting in microbial composition, showing an increase in copiotrophic and a decrease in oligotrophic microorganisms, i.e. slow growing organisms that can live in an environment with very low levels of nutrients (Ma et al., 2016; Ramirez et al., 2012). Actinobacteria are also considered copiothropic microorganisms (Ramirez et al., 2012), and they were found more abundantly in conventional soils compared to those of organic farming (Bonanomi et al., 2016b; Li et al., 2012), probably due to their ability to degrade agrochemicals used in conventional farming (De Schrijver and De Mot, 1999). In our study, both Proteobacteria that Actinobacteria were more abundant in the treatments with synthetic fertilizer applications, but not when the soil had been previously fumigated.

Gemmatimonadetes and Acidobacteria showed a highest abundance in the treatments with the application of organic materials, in accordance to Chaudhry et al. (2012) and Ramirez et al. (2012). Some members of Gemmatimonadetes partially affect soil fertility being involved in the cycle of essential micro- or macro-nutrients (Chaudhry et al. 2012). Acidobacteria are generally considered oligotrophs and usually they flourish in natural ecosystems respect to cropland (Pershina et al., 2015), thanks to their ability to degrade recalcitrant organic compounds (Fierer et al., 2012). Compared to the other treatments, a very low abundance of Acidobacteria was observed in fumigated soil. This result can be explained by the agrochemical suppressive effects on the microbial populations, and the slow growth of the Acidobacteria. In contrast with Acidobacteria, Bacteroidetes abundance was very high in fumigated soils compared to the other treatments. Since Bacteroidetes are known for their ability to rapidly

exploit bioavailable organic matter (Acosta-Martínez et al., 2008), their greater abundance is probably due to the high presence of organic compounds originating from microorganisms killed by the soil fumigation.

Among eukarya, amoeba and fungi belonging to the *Nucletmycea* clade (Brown et al., 2009) are the dominant microorganisms across all treatments, whereas the abundance of *Amoebozoa* members and SAR super-group largely varied with the treatments. Amoeba are single-celled organisms that live in several environments and are considered as bioindicators in terrestrial environments (Foissner, 1999). Their abundance is significantly reduced in the conventional respect to organic farming soils (Foissner, 1999). Bonkowski and Brandt (2002) have found that members of *Amoebozoa*, such as *Acanthamoeba*, affect the composition and function of rhizosphere microorganisms. In particular, their results showed that amoebae selectively stimulate certain bacterial strains capable of promoting plant growth through the release of hormonal substances such as indolyl-3-acetic acid. In accordance to Hartmann et al. (2015), the use of organic amendments also increases the number of species and richness of fungi communities. However, a different response was observed depending on the type of organic material applied. Bonanomi et al. (2016a) have also reported that eukarya composition in soil was significantly affected by organic farming compared to conventional cultivation. They suggest that eukarya play a key role in plant growth and decomposition processes of organic materials.

5. Conclusions

The present study revealed that, compared with the use of synthetic fertilizers, application of organic materials improves soil physical and chemical properties, as well as microbial community composition, diversity and functionality. However, the effects largely depend on the type and application frequency of organic amendments. The treatment that provides organic N and labile C (i.e., alfalfa plus glucose) promotes soil fertility and sustains plant growth, and therefore can be considered as a suitable alternative to the use of synthetic fertilizers. We acknowledge the limitations of this study that was carried out by using only one soil type, one target species, and a limited sample size for microbiological analysis. Then, further studies are needed to confirm and extend the trends reported in this experiment. Moreover, the limited knowledge of the mechanisms behind the interactions among different organic materials indicates the urgent need for further studies on this issue to identify and develop organic

amendment combinations and timing of application that maximize plant productivity in different agricultural systems.

6. References

Acosta-Martínez, V., Dowd, S., Sun, Y., Allen, V., 2008. Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. Soil Biology and Biochemistry 40, 2762–2770. doi:10.1016/j.soilbio.2008.07.022

Bartelt-Ryser, J., Joshi, J., Schmid, B., Brandl, H., Balser, T., 2005. Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. Perspectives in Plant Ecology Evolution and Systematics 7, 27–49. doi:10.1016/j.ppees.2004.11.002

Bastida, F., Kandeler, E., Moreno, J.L., Ros, M., García, C., Hernández, T., 2008. Application of fresh and composted organic wastes modifies structure, size and activity of soil microbial community under semiarid climate. Applied Soil Ecology 40, 318–329. doi:10.1016/j.apsoil.2008.05.007

Bastida, F., Selevsek, N., Torres, I.F., Hernández, T., García, C., 2015. Soil restoration with organic amendments: linking cellular functionality and ecosystem processes 5, 15550.

Belasco, E., Galinato, S., Marsh, T., Miles, C., Wallace, R., Ligon, E., 2013. High Tunnels Are My Crop Insurance: An Assessment of Risk Management Tools for Small-scale Specialty Crop Producers. Agricultural and Resource Economics Review 42, 403–418. doi:10.1017/S1068280500004445

Berry, B.M., Sylvester-Bradley, R., Philipp, L., Hatch, D.J., Cuttle, S.P., Rayns, F.W., Gosling, P., 2002. Is the productivity of organic farms restricted by the supply of available nitrogen? Soil Use and Management 18, 248–255. doi:10.1079/SUM2002129

Bonanomi, G., Antignani, V., Pane, C., Scala, F., 2007. Supression of Soilborne Fungal Diseases with Organic Amendments. Journal of Plant Pathology 89, 311–324. doi:10.2307/41998410

Bonanomi, G., D'Ascoli, R., Antignani, V., Capodilupo, M., Cozzolino, L., Marzaioli, R., Puopolo, G., Rutigliano, F.A., Scelza, R., Scotti, R., Rao, M.A., Zoina, A., 2011. Assessing soil quality under intensive cultivation and tree orchards in Southern Italy. Applied Soil Ecology 47, 184–194. doi:10.1016/j.apsoil.2010.12.007

Bonanomi, G., D'Ascoli, R., Scotti, R., Gaglione, S.A., Caceres, M.G., Sultana, S., Scelza, R., Rao, M.A., Zoina, A., 2014. Soil quality recovery and crop yield enhancement by combined application of compost and wood to vegetables grown under plastic tunnels. Agriculture, Ecosystems and Environment 192, 1–7. doi:10.1016/j.agee.2014.03.029

Bonanomi, G., De Filippis, F., Cesarano, G., La Storia, A., Ercolini, D., Scala, F., 2016a. Organic farming induces changes in soil microbiota that affect agro-ecosystem functions. Soil Biology and Biochemistry 103, 327–336. doi:10.1016/j.soilbio.2016.09.005

Bonanomi, G., Gaglione, S.A., Cesarano, G., Sarker, T.C., Pascale, M., Scala, F., Zoina, A., 2016b. Frequent Application of Organic Matter to Agricultural Soil Increases Fungistasis. doi:10.1016/S1002-0160(15)600

Bonilla, N., Gutiérrez-Barranquero, J.A., De Vicente, A., Cazorla, F.M., 2012. Enhancing soil quality and plant health through suppressive organic amendments. Diversity 4, 475–491. doi:10.3390/d4040475

Bonkowski, M., Brandt, F., 2002. Do soil protozoa enhance plant growth by hormonal effects? Soil Biology and Biochemistry 34, 1709–1715. doi:10.1016/S0038-0717(02)00157-8

Bronick, C., Lal, R., 2005. Soil structure and management: a review. Geoderma 124, 3–22. doi:10.1016/j.geoderma.2004.03.005

Bronwyn, D.H., Raymond, L.C., Wendy, M., Clive, A.K., Clive, E.P., 1997. Using the Gini coefficient with BIOLOG substrate utilisation data to provide an alternative quantitative measure for comparing bacterial soil communities. Journal of Microbiological Methods 30, 91–101. doi:10.1016/S0167-7012(97)00048-1

Brown, M.W., Spiegel, F.W., Silberman, J.D., 2009. Phylogeny of the "forgotten" cellular slime mold, Fonticula alba, reveals a key evolutionary branch within Opisthokonta. Molecular Biology and Evolution 26, 2699–2709. doi:10.1093/molbev/msp185

Bulluck, L.R., Brosius, M., Evanylo, G.K., Ristaino, J.B., 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. Applied Soil Ecology 19, 147–160. doi:10.1016/S0929-1393(01)00187-1

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature methods 7, 335–6. doi:10.1038/nmeth.f.303

Chaudhry, V., Rehman, A., Mishra, A., Chauhan, P.S., Nautiyal, C.S., 2012. Changes in Bacterial Community Structure of Agricultural Land Due to Long-Term Organic and Chemical Amendments. Microbial Ecology 64, 450–460. doi:10.1007/s00248-012-0025-y

Croteau, G.A., Zibilske, L.M., 1998. Influence of papermill processing residuals on saprophytic growth and disease caused by Rhizoctonia solani. Applied Soil Ecology 10, 103–115.

De Schrijver, a, De Mot, R., 1999. Degradation of pesticides by actinomycetes. Critical reviews in microbiology 25, 85–119. doi:10.1080/10408419991299194

Diacono, M., Montemurro, F., 2010. Long-term effects of organic amendments on soil fertility. A review. Agronomy for Sustainable Development 30, 401–422.

Dick, R.P.., Rasmussen, P.E.., Kerle, E.A.., 1988. Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. Biology and Fertility of Soils 6, 159–164. doi:10.1007/BF00257667

Duong, T.T.T., Baumann, K., Marschner, P., 2009. Frequent addition of wheat straw residues to soil enhances carbon mineralization rate. Soil Biology and Biochemistry 41, 1475–1482. doi:10.1016/j.soilbio.2009.04.003

Ercolini, D., De Filippis, F., La Storia, A., Iacono, M., 2012. "Remake" by high-throughput sequencing of the microbiota involved in the production of water Buffalo mozzarella cheese. Applied and Environmental Microbiology 78, 8142–8145. doi:10.1128/AEM.02218-12

Ferreras, L., Gomez, E., Toresani, S., Firpo, I., Rotondo, R., 2006. Effect of organic amendments on some physical, chemical and biological properties in a horticultural soil. Bioresource Technology 97, 635–640. doi:10.1016/j.biortech.2005.03.018

Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. The ISME Journal 6, 1007–17. doi:10.1038/ismej.2011.159

Foissner, W., 1999. Soil Protozoa As Bioindicator: Pros And Cons, Methods, Diversity, Rpresentative Examples. Agriculture, Ecosystems & Environment 74, 95–112.

García-Gil, J.C., Plaza, C., Soler-Rovira, P., Polo, A., 2000. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biology and Biochemistry 32, 1907–1913. doi:10.1016/S0038-0717(00)00165-6

Garland, J.L., Mills, A.L., 1991. Classification and characterisation of heterotrophic microbial communities on the basis of pattern of community-level sole-carbon-source utilization. Applied and environmental microbiology 57, 2351–2359.

Hartmann, M., Frey, B., Mayer, J., Mader, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. ISME J 9, 1177–1194.

Haynes, R.J., Naidu, R., 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: A review. Nutrient Cycling in Agroecosystems 51, 123–137. doi:10.1023/A:1009738307837

Hedley, M., Bolan, N., 2003. Role of Carbon, Nitrogen, and Sulfur Cycles in Soil Acidification, in: Handbook of Soil Acidity. CRC Press. doi:doi:10.1201/9780203912317.ch2

Himmelstein, J.C., Maul, J.E., Everts, K.L., 2014. Impact of Five Cover Crop Green Manures and Actinovate on Fusarium Wilt of Watermelon. Plant Disease 98, 965–972. doi:10.1094/PDIS-06-13-0585-RE

Jones, D.L., Rousk, J., Edwards-Jones, G., DeLuca, T.H., Murphy, D. V., 2012. Biochar-mediated changes in soil quality and plant growth in a three year field trial. Soil Biology and Biochemistry 45, 113–124. doi:10.1016/j.soilbio.2011.10.012

Ju, X.T., Kou, C.L., Christie, P., Dou, Z.X., Zhang, F.S., 2007. Changes in the soil environment from excessive application of fertilizers and manures to two contrasting intensive cropping systems on the North China Plain. Environmental Pollution 145, 497–506. doi:10.1016/j.envpol.2006.04.017

Kandeler, E., Stemmer, M., Klimanek, E.-M., 1999. Response of soil microbial biomass, urease and xylanase within particle size fractions to long-term soil management. Soil Biology and Biochemistry 31, 261–273. doi:10.1016/S0038-0717(98)00115-1

Kemper, W.D., Rosenau, R.C., 1986. Aggregate Stability and Size Distribution. Methods of soil analysis. Part 1. Physical and mineralogical methods 9, 425–442.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota - A review. Soil Biology and Biochemistry 43, 1812–1836. doi:10.1016/j.soilbio.2011.04.022

Li, R., Khafipour, E., Krause, D.O., Entz, M.H., de Kievit, T.R., Fernando, W.G.D., 2012. Pyrosequencing Reveals the Influence of Organic and Conventional Farming Systems on Bacterial Communities. PLoS ONE 7. doi:10.1371/journal.pone.0051897

Liang, C.H., Tang, Y., Xu, X.C., Hui, C.Z., 1997. Dynamics of soil salt in protected vegetable fields in Shenyang, China. Soil Fertility and Fertilizer Recommendation in Vegetable Fields, Hehai University Publisher, Nanjing,.

Ma, N., Zhang, L., Zhang, Y., Yang, L., Yu, C., Yin, G., Doane, T.A., Wu, Z., Zhu, P., Ma, X., 2016. Biochar improves soil aggregate stability and water availability in a mollisol after three years of field application. PLoS ONE 11, 1–10. doi:10.1371/journal.pone.0154091

Mallory, E.B., Griffin, T.S., 2007. Impacts of Soil Amendment History on Nitrogen Availability from Manure and Fertilizer. Soil Science Society of America Journal 71, 964. doi:10.2136/sssaj2006.0244

Marschner, H., 1995. Mineral Nutrition of Higher Plants, Mineral Nutrition of Higher Plants. Elsevier. doi:10.1016/B978-012473542-2/50004-3

Marschner, P., Kandeler, E., Marschner, B., 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biology and Biochemistry 35, 453–461. doi:10.1016/S0038-0717(02)00297-3

Martini, E.A., Buyer, J.S., Bryant, D.C., Hartz, T.K., Denison, R.F., 2004. Yield increases during the organic transition: Improving soil quality or increasing experience? Field Crops Research 86, 255–266. doi:10.1016/j.fcr.2003.09.002

McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Andersen, G.L., Knight, R., Hugenholtz, P., 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME journal 6, 610–8.

Melero, S., Porras, J.C.R., Herencia, J.F., Madejon, E., 2006. Chemical and biochemical properties in a silty loam soil under conventional and organic management. Soil and Tillage Research 90, 162–170. doi:10.1016/j.still.2005.08.016

Nett, L., Ruppel, S., Ruehlmann, J., George, E., Fink, M., 2012. Influence of Soil Amendment History on Decomposition of Recently Applied Organic Amendments. Soil Science Society of America Journal 76, 1290. doi:10.2136/sssaj2011.0190

Nielsen, M.N., Winding, A., 2002. Microorganisms as indicators of soil health., NERI technical report.

Noble, R., Coventry, E., 2005. Suppression of soil-borne plant diseases with composts: A review. Biocontrol Science and Technology 15, 3–20. doi:10.1080/09583150400015904

Pershina, E., Valkonen, J., Kurki, P., Ivanova, E., Chirak, E., Korvigo, I., Provorov, N., Andronov, E., 2015. Comparative analysis of prokaryotic communities associated with organic and conventional farming systems. PLoS ONE 10, 1–16. doi:10.1371/journal.pone.0145072

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Research 41. doi:10.1093/nar/gks1219

Ramirez, K.S., Craine, J.M., Fierer, N., 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology 18, 1918–1927. doi:10.1111/j.1365-2486.2012.02639.x

Ros, M., Klammer, S., Knapp, B., Aichberger, K., Insam, H., 2006. Long-term effects of compost amendment of soil on functional and structural diversity and microbial activity. Soil Use and Management 22, 209–218. doi:10.1111/j.1475-2743.2006.00027.x

Santamaria, P., Elia, a., Parente, a., Serio, F., 1998. Fertilization strategies for lowering nitrate content in leafy vegetables: chicory and rocket salad cases. Journal of Plant Nutrition 21, 1791–1803. doi:10.1080/01904169809365524

Scarascia-Mugnozza, G., Sica, C., Russo, G., 2012. Plastic Materials in European Agriculture: Actual Use and Perspectives. Journal of Agricultural Engineering 42, 15–28. doi:10.4081/jae.2011.3.15

Seufert, V., Ramankutty, N., Foley, J.A., 2012. Comparing the yields of organic and conventional agriculture. Nature 485, 229–232. doi:10.1038/nature11069

Spaccini, R., Mbagwu, J.S.C., Igwe, C.A., Conte, P., Piccolo, A., 2004. Carbohydrates and aggregation in lowland soils of Nigeria as influenced by organic inputs. Soil and Tillage Research 75, 161–172. doi:10.1016/S0167-1987(03)00158-2

Sparks, D.L., 1996. Methods of Soil Analysis Part 3—Chemical Methods. Methods of Soil Analysis Part 3—Chemical Methods i–xxi. doi:10.2136/sssabookser5.3.frontmatter

Stark, C.H., Condron, L.M., O'Callaghan, M., Stewart, A., Di, H.J., 2008. Differences in soil enzyme activities, microbial community structure and short-term nitrogen mineralisation resulting from farm management history and organic matter amendments. Soil Biology and Biochemistry 40, 1352–1363. doi:10.1016/j.soilbio.2007.09.025

Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., De MacÊdo, J.L.V., Blum, W.E.H., Zech, W., 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. Plant and Soil 291, 275–290. doi:10.1007/s11104-007-9193-9

Stockdale, E., Shepherd, M., Fortune, S., Cuttle, S., 2002. Soil fertility in organic farming systems – fundamentally different? Soil Use and Management 18, 301–308. doi:10.1079/SUM2002143

Tejada, M., Hernandez, M.T., Garcia, C., 2009. Soil restoration using composted plant residues: Effects on soil properties. Soil and Tillage Research 102, 109–117.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nature 418, 671–7. doi:10.1038/nature01014

Torres, I.F., Bastida, F., Hernández, T., García, C., 2015. The effects of fresh and stabilized pruning wastes on the biomass, structure and activity of the soil microbial community in a semiarid climate. Applied Soil Ecology 89, 1–9.

Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and proposed modification of the chromic acid titration method. Soil Science 37, 29–38. doi:10.1097/00010694-193401000-00003

Xue, J.C., Bi, D.Y., Li, J.J., Yin, Y.X., Wu, Z.H., 1994. The physiological problems caused by soil factors in protected vegetable fields. Soil and Fertilizer 1, 4–9.

Figures and tables

Fig. 1. Cumulated crop biomass of 6 cropping cycle expressed as g of dry mass per m^{-2} $year^{-1}$. Data refer to mean of three replicates ± standard deviation. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively. Different letters indicate statistically significant differences between different treatments (Duncan's test at $p < 0.05$).

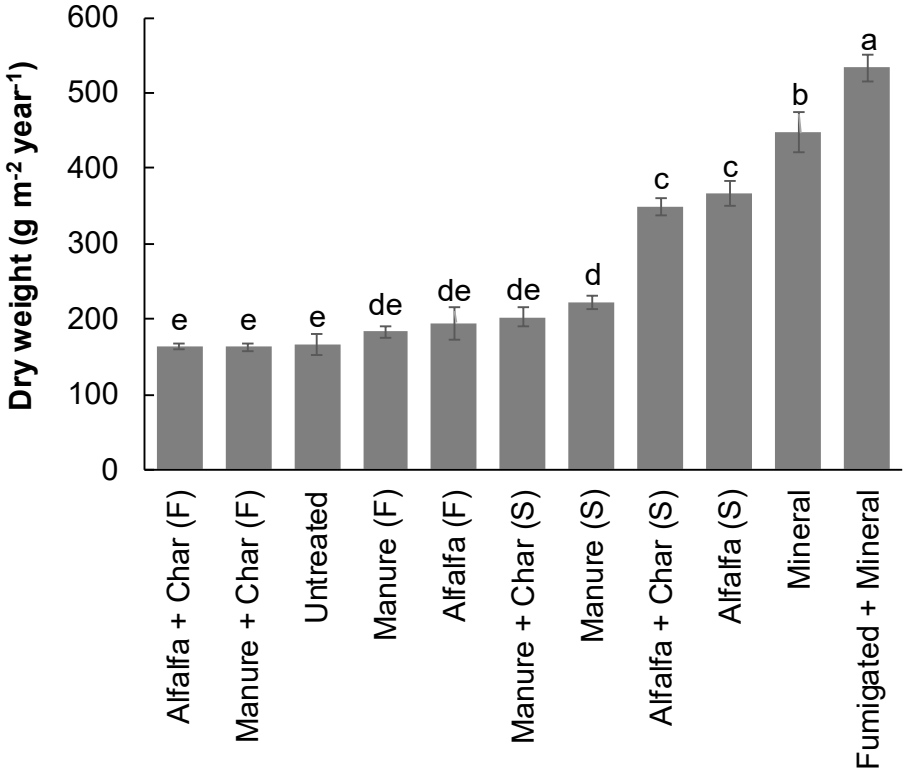

Fig. 2. Percent distribution of water-stable aggregates of different size (mm) in relation to soil treatments. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

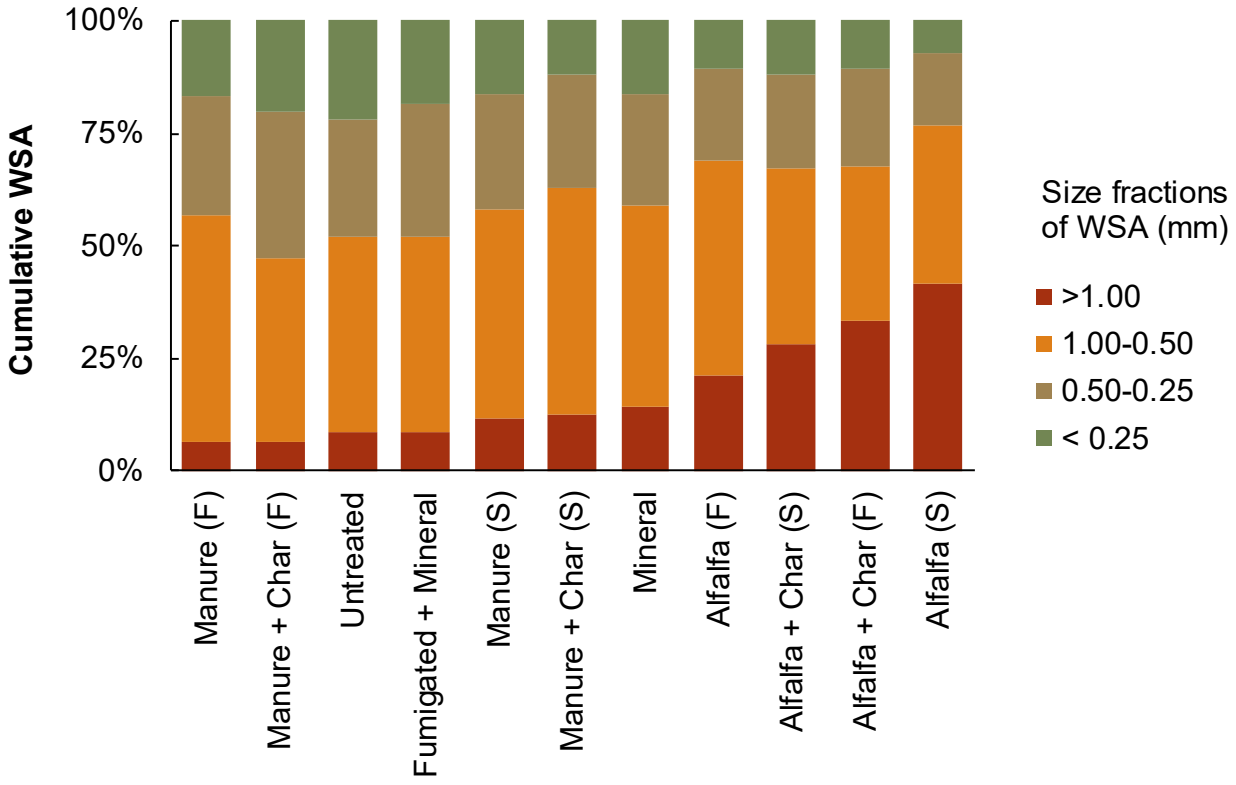

Fig. 3. Histograms showing A) number of observed OTUs, B) Chao1 richness and C) Shannon diversity index based on bacteria (left side) and eukarya (right side) communities. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

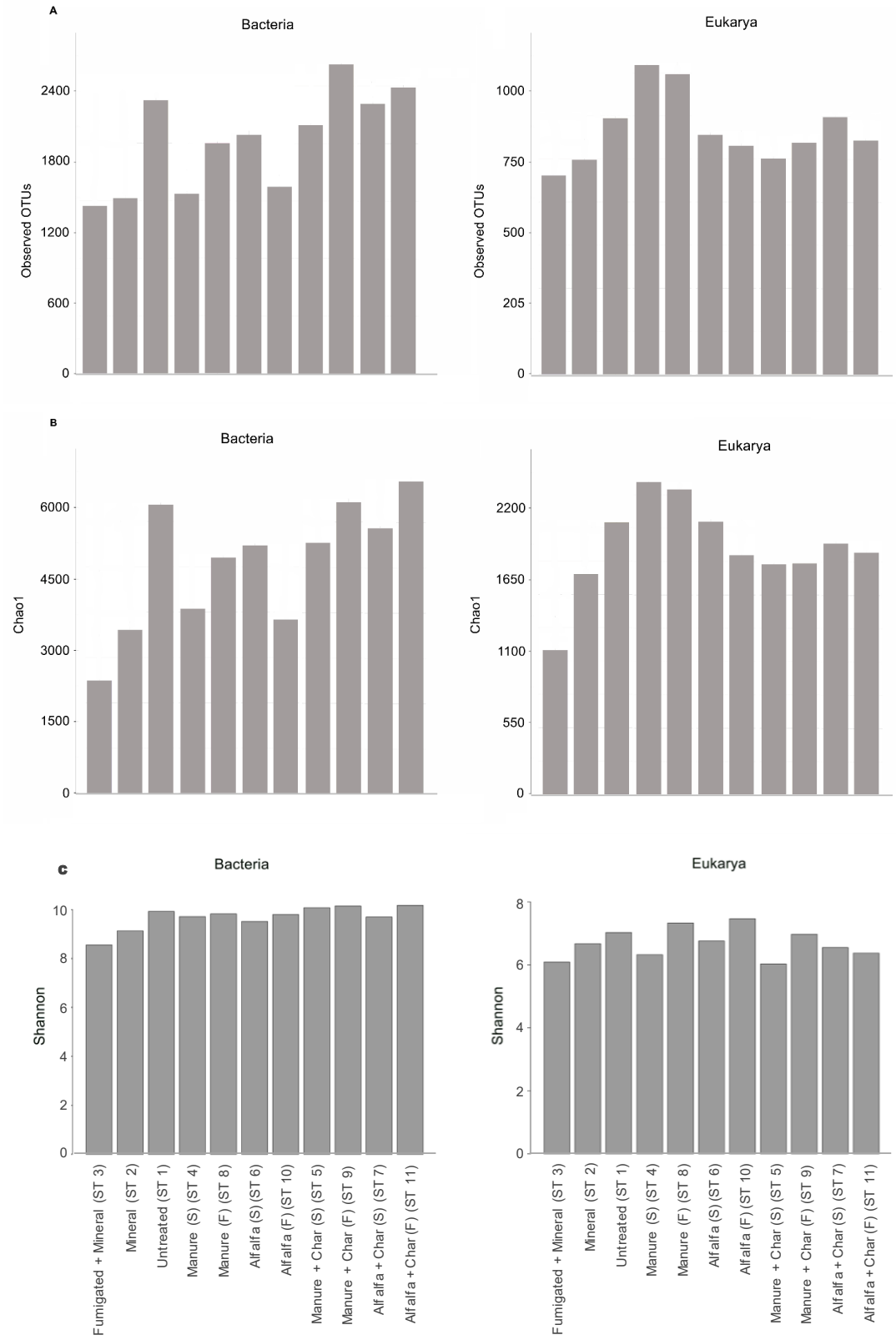

Fig. 4. Bacterial (a) and eukaryotic (b) composition of the soil samples analysed in this study. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

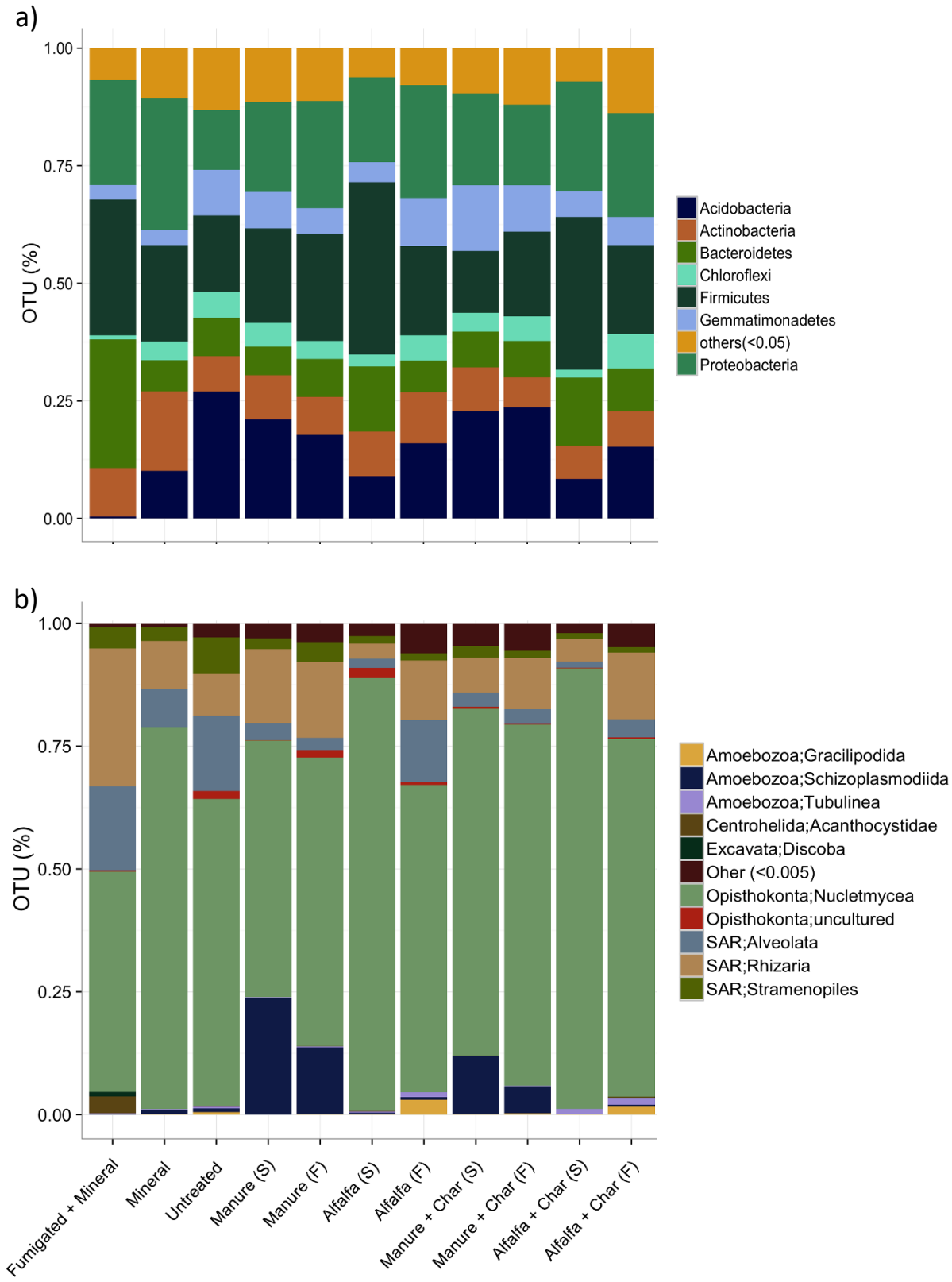

Fig. S1. Pictures of the experiment system: (a) organic materials used as amendments; (b1) addition of the organic materials, and (b2) the soil after mixing with the organic amendment; (b3) germination and (b4) pre-harvesting phases of the rocket (*Eruca sativa*) cultivation; (c) panoramic of the mesocosms with the irrigation system.

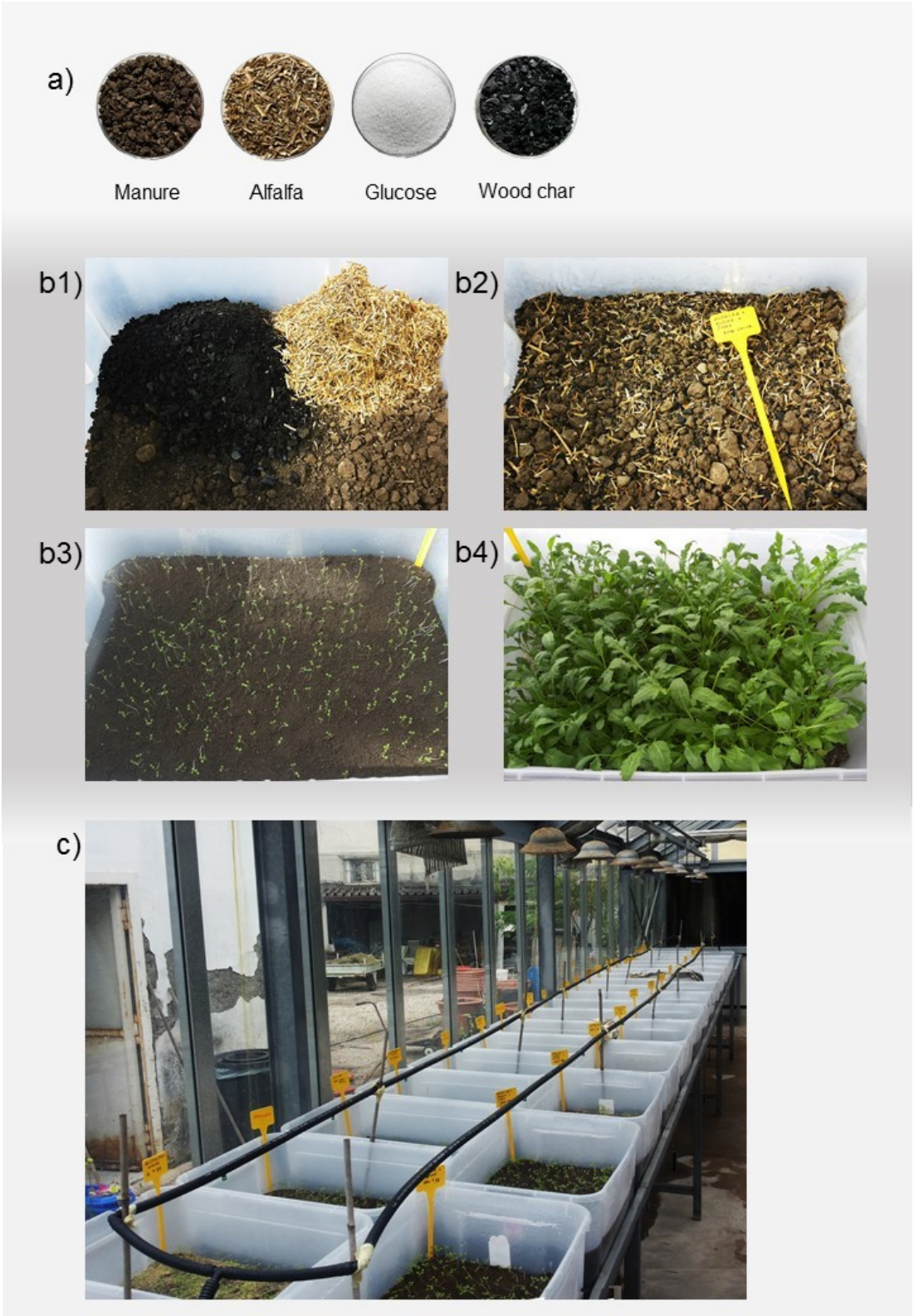

Fig. S2. AWCD recorded for the six main chemical groups in different soil treatments. Absorbance readings at 590 nm after 96 hours of incubation. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

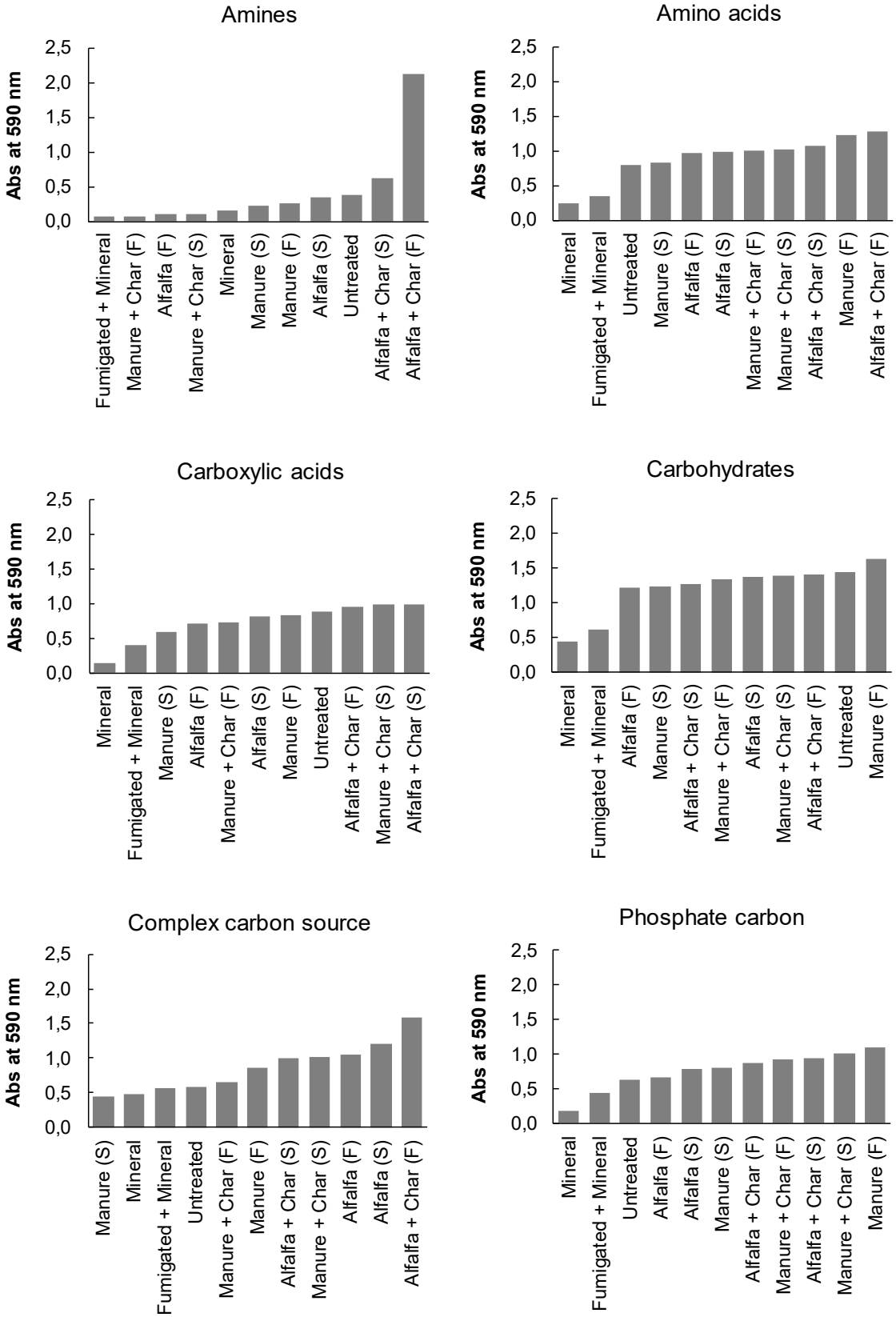

Fig. S3. Fungi composition at genus level in soils derived from different treatments. Only fungal genera present with abundance higher than 0.3% in at least one sample are shown. Low abundance genera are summed up as "Other Fungi". Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

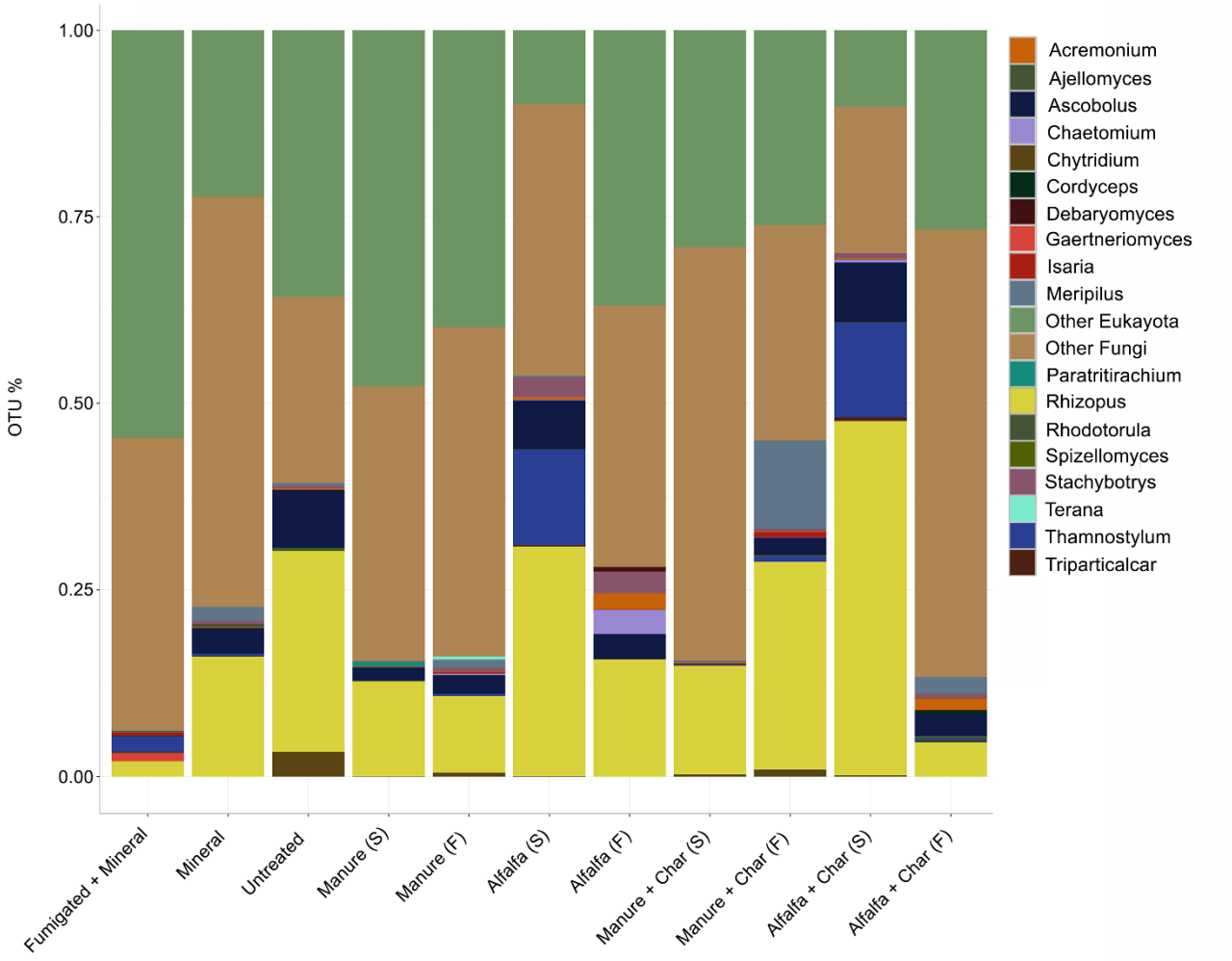

Fig. S4. Principal Component Analysis (PCA) based on the bacterial (a) and eukaryotic (b) community composition at genus level. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

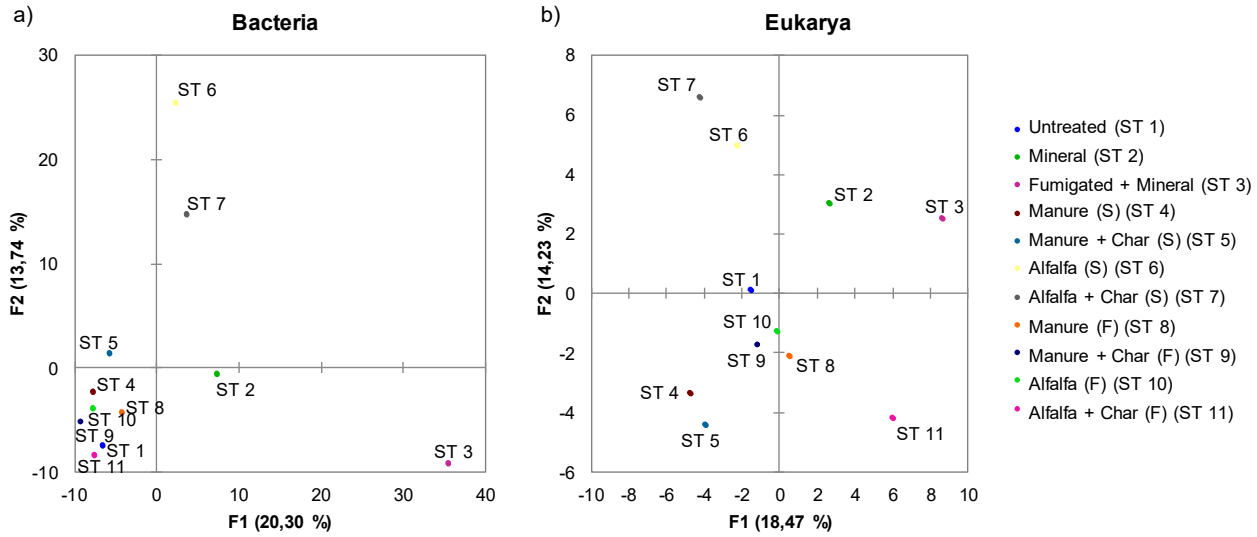

Fig. S5. Hierarchical average-linkage clustering of the samples based on the Pearson's correlation coefficient of the abundance of bacterial genera showing an abundance of at least 0.1%. The color scale represents the scaled abundance of each variable, denoted as Z-score, with red indicating high abundance and blue indicating low abundance. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

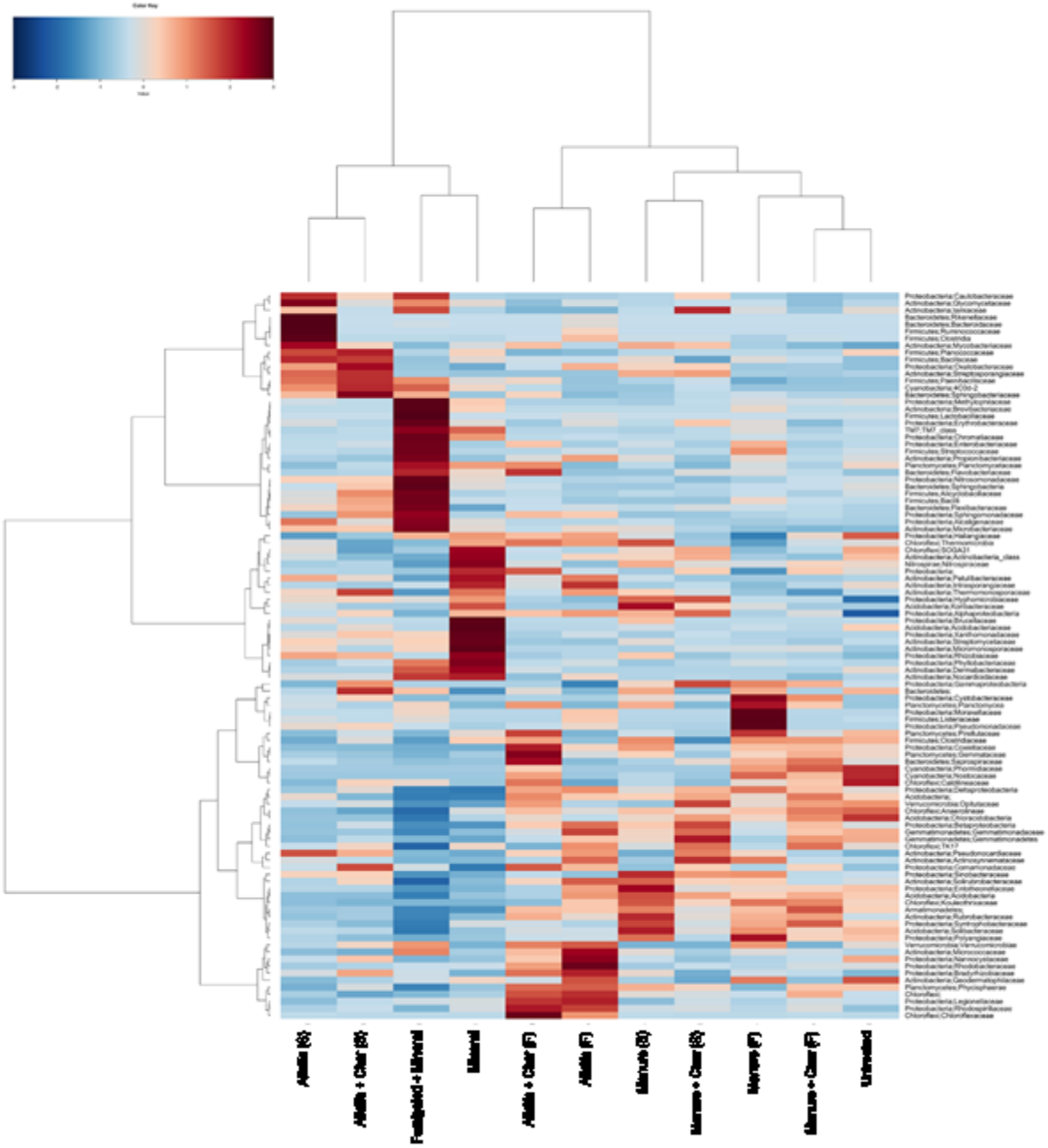

Fig. S6. Hierarchical average-linkage clustering of the samples based on the Pearson's correlation coefficient of the abundance of eukarya genera. The color scale represents the scaled abundance of each variable, denoted as Z-score, with red indicating high abundance and blue indicating low abundance. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

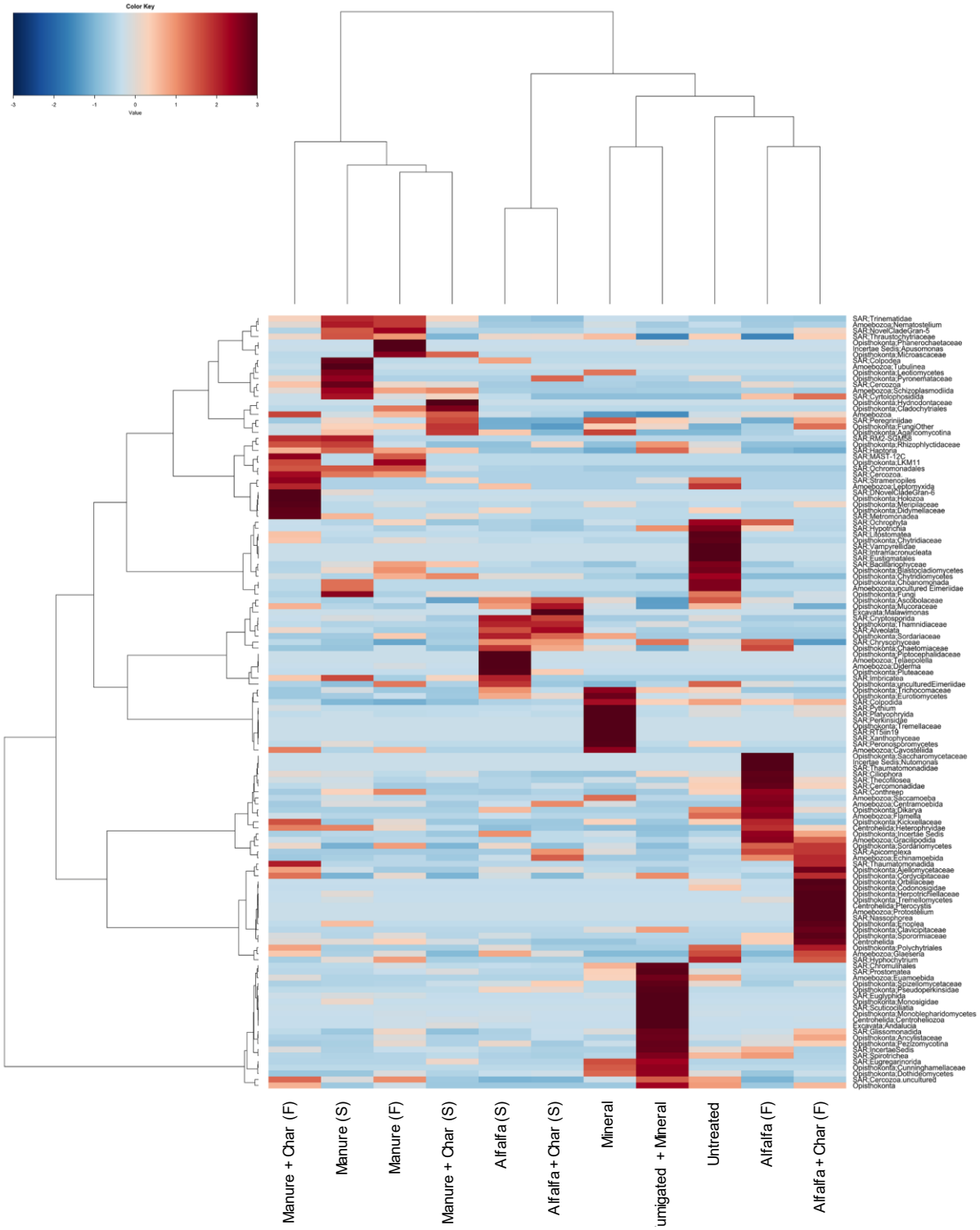

Table 1

Summary of 11 soil treatments with details about application frequencies of organic matter (t ha^{-1}) and mineral nutrients (kg ha^{-1}).

Code	Treatment	Organic matter (t ha^{-1} $year^{-1}$)	Mineral fertilizer (kg ha^{-1} $year^{-1}$)	Application frequency (n° $year^{-1}$)
ST 1	Untreated			
ST 2	Mineral		Ammonium sulphate (100) Calcium nitrate (40) Urea (40)	Weekly applications (35) ‡
ST 3	Fumigated + Mineral		Ammonium sulphate (100) Calcium nitrate (40) Urea (40)	Weekly applications (35) ‡
ST 4	Manure (S)	Compost manure (15)		Single application (1) §
ST 5	Manure + Char (S)	Compost manure (15) Wood biochar (30) †		Single application (1) §
ST 6	Alfalfa (S)	Alfalfa straw (13) Glucose (7)		Single application (1) §
ST 7	Alfalfa + Char (S)	Alfalfa straw (13) Glucose (7) Wood biochar (30) †		Single application (1) §
ST 8	Manure (F)	Compost manure (15)		Weekly applications (35) ‡
ST 9	Manure + Char (F)	Compost manure (15) Wood biochar (30) †		Weekly applications (35) ‡
ST 10	Alfalfa (F)	Alfalfa straw (13) Glucose (7)		Weekly applications (35) ‡
ST 11	Alfalfa + Char (F)	Alfalfa straw (13) Glucose (7) Wood biochar (30) †		Weekly applications (35) ‡

† Wood biochar was applied at the start of incubation period
‡ Weekly applications of organic materials were added as a liquid extract
§ Single application of organic material was incorporated into the soil as powdered material
(S) Single, high dose application. (F) Frequent, low dose applications

Table 2

Physical, chemical and microbiological parameters under different soil treatments.

	Untreated (ST 1)	Mineral (ST 2)	Fumigated + Mineral (ST 3)	Manure (S) (ST 4)	Manure + Char (S) (ST 5)	Alfalfa (S) (ST 6)	Alfalfa + Char (S) (ST 7)	Manure (F) (ST 8)	Manure + Char (F) (ST 9)	Alfalfa (F) (ST 10)	Alfalfa + Char (F) (ST 11)
Physical & Chemical											
pH	7.26 ± 0.1 a	4.39 ± 0.11 d	5.38 ± 0.05 c	7.01 ± 0.11 b	7.02 ± 0.12 b	7.08 ± 0.1 ab	7.02 ± 0.08 b	7.2 ± 0.05 a	7.25 ± 0.1 a	7.16 ± 0.06 ab	7.17 ± 0.1 ab
EC (µS cm^{-1})	286 ± 20 f	2560 ± 60 a	1990 ± 70 b	329 ± 10 def	376 ± 5 cd	402 ± 8 c	363 ± 9 cde	318 ± 12 ef	293 ± 14 f	374 ± 11 cd	418 ± 10 c
$N\text{-}NO_3^-$ (mg l^{-1})	6.81 ± 0.76 b	350.67 ± 33.01 a	352.0 ± 6.0 a	10.61 ± 1.36 b	5.65 ± 4.46 b	6.23 ± 2.5 b	7.36 ± 3.97 b	8.54 ± 6.31 b	7.12 ± 2.84 b	5.23 ± 1.75 b	15.73 ± 3.97 b
$N\text{-}NH_4^+$ (mg l^{-1})	0.13 ± 0.08c	26.47 ± 13.7 a	18.43 ± 2.62 b	0.05 ± 0.02 c	0.04 ± 0.05 c	0.12 ± 0.04 c	0.22 ± 0.1 c	0.06 ± 0.05 c	0.04 ± 0.01 c	0.03 ± 0.02 c	0.22 ± 0.04 c
Organic carbon (g kg^{-1})	14.9 ± 0.1 g	14.45 ± 0.05 g	14.35 ± 0.05 g	26.43 ± 0.25 a	25.67 ± 0.45 a	23.5 ± 0.2 b	21.53 ± 1.2 c	17.03 ± 0.15 ef	16.87 ± 0.15 f	17.7 ± 0.1 e	18.67 ± 0.65 d
Soil aggregation (MWD)	0.58 ± 0.04 ef	0.66 ± 0.02 d	0.59 ± 0.04 ef	0.64 ± 0.04 de	0.68 ± 0.05 d	0.96 ± 0.03 a	0.81 ± 0.05 bc	0.59 ± 0.03 ef	0.55 ± 0.02 f	0.77 ± 0.03 c	0.85 ± 0.04 b
Microbiological											
Biolog EcoPlates™ (AWCD)	0.88 ± 0.1 bc	0.27 ± 0.09 d	0.43 ± 0.16 d	0.73 ± 0.12 c	1.01 ± 0.09 b	0.97 ± 0.11 b	1.01 ± 0.08 b	1.05 ± 0.18 ab	0.86 ± 0.12 bc	0.86 ± 0.06 bc	1.24 ± 0.09 a

Means of three replicates ± standard deviations. Different letter within each row indicate significant differences (Duncan test, $p < 0.05$).
(S) Single, high dose application. (F) Frequent, low dose applications.

Table 3

Cross correlation matrix between crop yield, soil properties and microbiological parameters

	Crop yield	**pH**	**EC**	**N-NO_3^-**	**N-NH_4^+**	**Organic carbon**	**Soil aggregation (MWD)**
pH	-0.78**						
EC	0.79**	-0.99**					
N-NO_3^-	0.81**	-0.96**	0.98**				
N-NH_4^+	0.69**	-0.93**	0.93**	0.89**			
Organic carbon	-0.22	0.44*	-0.51**	-0.53**	-0.49**		
Soil aggregation (MWD)	0.10	0.20	-0.20	-0.27	-0.21	0.42*	
Biolog EcoPlates™ (AWCD)	-0.66**	0.84**	-0.82**	-0.82**	-0.79**	0.39*	0.41*

Values are Pearson coefficients. Significant differences at the 0.05 (*) and 0.01 (**) are reported.

Table 4

Pearson's correlations between bacterial OTUs collapsed at phylum level, Chao1 and Shannon indices with soil properties and crop yield.

	Acidobacteria	Actinobacteria	Bacteroidetes	Chloroflexi	Firmicutes	Gemmatimonadetes	Proteobacteria	Chao1	Shannon
Crop yield	-0.88**	0.57	0.74**	-0.80**	0.63*	-0.71*	0.46	-0.67*	-0.94**
pH	0.58	-0.85**	-0.33	0.39	-0.12	0.55	-0.60	0.68*	0.79**
EC	-0.61*	0.82**	0.39	-0.39	0.12	-0.57	0.58	-0.68*	-0.82**
Organic carbon	0.23	-0.43	-0.21	0.11	-0.13	0.46	-0.07	0.59	0.59
N-NO_3^-	-0.64*	0.73*	0.51	-0.44	0.15	-0.58	0.52	-0.72*	-0.87**
N-NH_4^+	-0.58	0.82**	0.36	-0.37	0.10	-0.56	0.57	-0.67*	-0.80**
Soil aggregation (MWD)	-0.37	0.00	0.05	-0.04	0.53	-0.23	0.19	0.22	0.10
Biolog EcoPlates™ (AWCD)	0.40	-0.77**	-0.28	0.39	-0.05	0.38	-0.35	0.79**	0.79**

Values are Pearson coefficients. Significant differences at the 0.05 (*) and 0.01 (**) are reported.

Table S1

Chemical and physical properties of soil used in this study

Parameters		Value
pH		7.74
EC	dS m^{-1}	0.32
Total $CaCO_3$	g Kg^{-1}	7.16
Organic carbon	g Kg^{-1}	15.4
Organic matter	g Kg^{-1}	26.5
Total N	g Kg^{-1}	1.60
C/N		9.60
Available phosphorus (P_2O_5)	mg Kg^{-1}	239
Cation exchange capacity	meq 100 g^{-1}	36.3
Exchangeable potassium	meq 100 g^{-1}	1.81
Exchangeable magnesium	meq 100 g^{-1}	6.55
Exchangeable calcium	meq 100 g^{-1}	27.03
Exchangeable sodium	meq 100 g^{-1}	0.94

Table S2

Pearson's correlations between fungal OTUs collapsed at genus level, Chao1 and Shannon indices with soil properties and crop yield. Only fungi with significant correlation values ($p \leq 0.05$) are reported.

	Ascobolaceae; Thecotheus	Chaetomiaceae; Humicola	Clavicipitaceae; Neotyphodium	Cunninghamellaceae; Cunninghamella	Incertae Sedis; Rhodotorula	Incertae Sedis; Stachybotrys	Incertae Sedis; Yarrowia	Piptocephalidaceae; Syncephalis	Spizellomycetaceae; Gaertneriomyces	Thamnidiaceae; Thamnostylum	Tremellaceae; Cryptococcus	Trichocomaceae; Aspergillus	Chao1	Shannon
Crop yield	-0.18	-0.13	0.78**	0.84**	0.59	-0.02	0.45	0.24	0.67*	0.40	0.45	0.70*	0.34	0.02
pH	0.11	0.23	-0.70*	-0.89**	-0.93**	0.24	-0.82**	0.13	-0.47	0.12	-0.82**	-0.80**	0.02	0.10
EC	-0.14	-0.27	0.76**	0.93**	0.91**	-0.22	0.78**	-0.13	0.54	-0.14	0.78**	0.78**	-0.03	-0.04
Organic carbon	0.60*	0.76**	-0.48	-0.51	-0.36	-0.18	-0.28	-0.09	-0.38	0.27	-0.28	-0.48	-0.05	0.06
N-NO_3^-	-0.15	-0.33	0.85**	0.98**	0.84**	-0.27	0.67*	-0.15	0.67*	-0.15	0.67*	0.71*	-0.03	-0.04
N-NH_4^+	-0.15	-0.29	0.73*	0.92**	0.92**	-0.24	0.80**	-0.14	0.51	-0.15	0.80**	0.79**	-0.01	-0.06
Soil aggregation (MWD)	-0.05	0.38	-0.30	-0.29	-0.12	0.66*	-0.10	0.66*	-0.26	0.67*	-0.10	0.07	0.26	0.32
Biolog EcoPlates™ (AWCD)	0.19	0.46	-0.68*	-0.83**	-0.76**	0.19	-0.68*	0.15	-0.48	0.20	-0.68*	-0.74**	0.04	0.34

Values are Pearson coefficients. Significant differences at the 0.05 (*) and 0.01 (**) are reported.

Chapter 4

LONG-TERM ORGANIC MANAGEMENT BOOSTS BENEFICIAL MICROBES POPULATION, AND IMPROVES SOIL FERTILITY AND CROP YIELD AS COMPARED TO LONG-TERM CONVENTIONAL MANAGEMENT

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Abstract

Long-term adoption of conventional agricultural system negatively affects soil fertility and can compromise the quality and quantity of crop production. To avoid these problems, application of organic materials has been proposed as a valid and effective strategy. In the present study, long-term effects of 11 soil treatments on crop yield and quality, as well as on soil fertility were evaluated. Soil treatments included: two conventional managements, eight organic treatments based on different amendment type and application frequency, and one untreated soil as control. Crop quality was assessed by measuring the NO_3^- leaves content, whereas soil chemical and microbiological (i.e., total microbial activity and microbial community functioning) properties were evaluated to understand the effects on soil fertility. Finally, changes in soil bacterial microbiota was characterized by high-throughput sequencing of bacterial rRNA gene markers. In the long-term, application of organic amendment significantly improves crop yield, especially when alfalfa and glucose were applied as single dose. On the contrary, NO_3^- leaves content was low for manure application, independently by the frequency, and for repeated application of alfalfa and glucose. Application of synthetic fertilizer negatively affects soil chemical properties and reduce soil microbial activity and functionality, as well as richness and diversity of bacterial community. Compared to organic amendment, application of synthetic fertilizer strongly reduced the abundance of Acidobacteria, Chloroflexi and Nitrospirae, whereas the presence of Bacteroidetes and Proteobacteria was promoted. Finally, seedling establishment and plant survival was found to be higher with application of organic amendments than synthetic fertilizers. This study suggest that long-term application of organic material can effectively improve soil fertility and support plant growth.

Key Words: Organic amendment, Crop quality, Soil microbiota, Plant survival, Soil sickness.

1. Introduction

Intensive agriculture is a cultivation system characterized by high levels of input and output per unit of agricultural land area. Mechanization of agricultural practices, use of high yielding variety crops, applications of chemical fertilizers and agrochemicals allow to increase the crop yield with consequent economic benefit for the farmers (Tilman 2002). Intensive agriculture is adopted for the cultivation of a wide range of agricultural species both in open field and in protected environment conditions, like greenhouse and plastic tunnel. In particular vegetable cultivation under protected environment is a growing agricultural sector (Scarascia-Mugnozza et al. 2012), since the presence of favourable climatic conditions allow a rapid plant growth and the possibility of cultivation during the whole year (Martínez-Blanco et al. 2011).

However, the adoption of intensive agriculture systems for a long period leads to a deterioration of physical, chemical and biological quality of soil (Bonanomi et al. 2011; Maksimovic and Ilin 2012; Zhou and Wu 2015), which in turn negatively affects crop yield and quality (Steiner et al. 2007; Zoran et al. 2014). In fact, the adoption of monoculture or short-rotation, intensive use of synthetic fertilizers and agrochemicals, continuous soil tillage and removal of crop residues as well as the complete rainfall restriction under covered condition are often related with the occurrence of soil sickness problems (Bennett et al. 2012), including deterioration of soil physical and chemical quality (Ju et al. 2007; Marschner et al. 2003), reduction of microbial biomass and activity (Mäder et al. 2002; O'donnell et al. 2001) and loss of natural soil suppression (Li et al. 2015). Moreover, the presence of high nitrate (NO_3^-) soil content due to the excessive use of fertilizers leads to the accumulation of this compound in the leaves of vegetables like rocket, lettuce and spinach and, consequently, represents a hazard to the health of consumers (Fontes et al. 1997).

A possible solution for these problems is the application of organic amendment (Stockdale et al. 2002). Many studies reported the beneficial effects that organic amendments like compost, green and animal manure, organic wastes and biochar have on soil properties, including the improvement of soil aggregation and available water holding capacity (Bronick and Lal 2005), increase in soil organic matter (Li et al. 2012), enhancement of microbial activity and biomass (Ros et al. 2006), and plant protection from soilborne pathogens due to soil suppressivness (Bonilla et al. 2012). In addition, the adoption of organic materials to support plant growth has been considered as a valid alternative to the use of synthetic fertilizers by many authors (Blaise 2006; Bulluck et al. 2002; Goldstein et al. 2004; Melero et al. 2006). However, the beneficial effects derived by the use of organic amendments largely depend on

the quality and quantity of organic material, as well as by the application frequency (Bonanomi et al. 2016b; Liu et al. 2009; Sun et al. 2014). Most of the researches focussed their attention on the impact that single annual applications of organic material have on crop productivity and/or soil properties (review in Diacono and Montemurro 2010). On the contrary, the few available studies concerning frequent applications considered the effects on soil processes and biological functions including carbon (C) and nitrogen (N) mineralization (Duong et al. 2009; Mallory and Griffin 2007), soil basal respiration (Nett et al. 2012), enzymatic activities (Stark et al. 2008), soil fungistasis (Bonanomi et al. 2016b) and microbial biomass (Fließbach et al. 2007). Therefore, no study addressed the impact that organic amendment type and application frequency have on crop yield, soil fertility and soil microbial community.

Within agroecosystem, soil microorganisms, mainly bacteria and fungi, are fundamental to maintain a good level of soil fertility since they are responsible for a multitude of biological functions (Mendes et al. 2013). In addition, some microorganisms like mycorrhizal fungi, nitrogen-fixing bacteria, plant growth-promoting rhizobacteria, *Pseudomonas fluorescens* and *Trichoderma* spp. play a fundamental role in supporting plant growth and protection toward soilborne pathogens (Bonilla et al. 2012). Therefore, the knowledge of the composition and diversity of soil microbiota represents an important aspect in order to understand the positive and negative effects that different agricultural managements (e.g., conventional and organic) have on the agroecosystem. In this regard, new molecular approaches based on DNA sequencing-by-synthesis was developed in the last decade. This method known as "high-throughput sequencing" has been largely used to assesses the composition and diversity of soil microbial community in soils subjected to conventional or organic agricultural systems (Bonanomi et al. 2016a; Chaudhry et al. 2012; Sugiyama et al. 2010).

In our study, a 2-year long mesocosm experiment was performed by conditioning a soil with 11 treatments, including conventional (i.e., use of synthetic fertilizers and fumigants) and organic (i.e., use of different organic amendments type and application frequency) management approaches. So, the effects on crop yield, quality (i.e., nitrate leaf content) and health of rocket (*Eruca sativa*), as well as on soil chemical properties and microbial community were evaluated.

2. Material and methods

2.1. Soil collection, organic material and mesocosm experiment

A second experimental year was performed using the same mesocosms (i.e., soil treatments) and the same experimental conditions reported in Chapter 3. Briefly, soil from a

farm located in the Piana del Sele, a fertile alluvial plain situated in Salerno (Southern Italy, 40°33'13"N, 14°57'22"E) was collected in spring 2013. Soil had a silt loam texture with 22.1% clay, 56.6% silt, 21.3% sand, pH 7.74, electrical conductivity (EC) 0.32 dS m^{-1}, organic C 15.4 g kg^{-1}, total N 1.6 g kg^{-1}, C/N ratio 9.6, total $CaCO_3$ 7.16 g kg^{-1}, available P (P_2O_5) 239 mg kg^{-1}, cation exchange capacity (CEC) 36.3 meq 100 g^{-1}, exchangeable K^+ 1.81 meq 100 g^{-1}, exchangeable Mg^{2+} 6.55 meq 100 g^{-1}, exchangeable Ca^{2+} 27.03 meq 100 g^{-1}, exchangeable Na^+ 0.94 meq 100 g^{-1} (see Chapter 3).

The farm adopted an intensive farming system by ~10 years, characterized by the monoculture of rocket (*Eruca sativa*) under plastic tunnel (height ~4 m), intensive tillage, application of mineral fertilizers and soil disinfestation with Metham-Na fumigant. To compare the effect of mineral fertilizers plus soil fumigant with the use of different organic amendment types and application frequencies on crop yield and quality, as well as the impact on chemical and biological quality of soil in the short and long term, a mesocom experiment was performed.

Four types of organic amendment with different properties were used, including:

i. alfalfa straw (*Medicago sativa*) (N content = 3.93 ± 2.16%; C/N ratio = 11.43 ± 2.98) as source of organic N and recalcitrant C, at rate of 13 t ha^{-1} $year^{-1}$;
ii. glucose (N content = 0.00; C/N ratio = ∞) as source of short term labile C for microbes, at rate of 7 t ha^{-1} $year^{-1}$;
iii. compost manure (N content = 3.13 ± 0.64%; C/N ratio = 13.09 ± 1.16) as source of organic N and recalcitrant C, at rate of 15 t ha^{-1} $year^{-1}$;
iv. wood biochar (N content = 0.51 ± 0.11%; C/N ratio = 149.61 ± 7.26) a stable carbon material to improve soil physical properties, at rate of 30 t ha^{-1} once at the start of the experiment.

Considering the properties of different organic materials, 11 soil treatments were performed as follows: ST 1 - untreated soil (control); ST 2 – soil treated with synthetic fertilizers; ST 3 - soil fumigated by Metham-Na and treated with synthetic fertilizers; ST 4 – soil with a high rate, single application of compost manure at the start of the experiment; ST 5 - soil with a high rate, single application of compost manure plus wood biochar at the start of the experiment; ST 6 – soil with a high rate, single application of glucose and alfalfa straw at the start of the experiment; ST 7 - soil with a high rate, single application of glucose and alfalfa straw plus wood biochar at the start the experiment; ST 8 - soil with low application rates of compost manure added weekly during crop growth; ST 9 - soil with low application rates of compost manure added weekly during crop growth plus wood biochar at the start of the

experiment; ST 10 - soil with low application rates of glucose and alfalfa straw added weekly during the whole experiment; ST 11 - soil with low application rates of glucose and alfalfa straw added weekly during the whole experiment plus wood biochar at the start of the experiment (see Chapter 3 for details). Single application consisted in the incorporation into the soil of powdered organic material once a year, whereas, to avoid soiling the plants with powder of organic material, frequent application was performed by spreading organic liquid extracts (1:2 w/v) on soil surface once a week.

Mesocosms, consisting in 32 L plastic tray filled with 35 kg of soil, were set up in greenhouse equipped with automatic control of temperature. The temperature was kept at 24 ± 4°C day and 18 ± 4°C night in spring and summer and 18 ± 4°C day and 12 ± 4°C in fall and winter.

2.2. Crop cultivation and yield

Four consecutive cycles of rocket (*Eruca sativa*) cultivation were performed during the second year of mesocosm experiment by mimicking the ordinary cultivation method used by farmers in Southern Italy. Briefly, 600 mg m^{-2} of rocket seeds were sown by hand spreading and covered with a thin layer (~3 mm) of soil. Mesocosms were irrigated every 3 days using a sprinkler irrigation system in order to maintain a soil moisture content between 65% and 85% of field capacity. The length of the cycle was approximately of 35 days in summer and 50 days in winter. At the end of each cycle, the plants were cut at ground level, air-dried in a dehydrator until constant weight was reached, then the aboveground biomass of each mesocosm was recorded. After the quantification, dried material was packed in separate bags and stored to assay the nitrate content in the leaves.

2.3. Seedling establishment and plant survival

During the two experimental years no agrochemicals (i.e., herbicides, fungicides and insecticides) were applied, except for ST 3 where soil fumigation was performed with Metham-Na prior to each cultivation cycle. Therefore, the composition and diversity of soil bacterial communities as well as the changes in soil chemical and biological properties resulting by the adoption of different soil treatments can affect seed germination and plant health. In this regard, seedlings establishment and plants survival were monitored during the fourth cycle of second experimental year. In detail, five plastic collars (Ø 6 cm; h 1.5 cm) were placed on soil surface

and 10 seeds were sown in each of them, for a total of 50 seeds for mesocosm. Then, the number of live plants was recorded weekly until the end of the cycle (50 days).

2.4. Nitrate content of leaves

Determination of nitrate (NO_3^-) content in rocket leaves was performed in order to assess the impact of the soil treatments on crop quality by using the method describe by Cataldi et al. (2003). Briefly, dried plant material was finely pulverized, suspended in Milli-Q water (5:1 w/v) and incubated for 10 minutes at 80° C in a thermostatic bath (ShakeTemp SW22, Julabo, Seelbach, Germany). Subsequently, the samples were centrifuged at 6000 rpm for 10 minutes and filtered with a 0.20 µm filter syringe to separate solid material from aqueous extract. NO_3^- content of aqueous extract was determined using a Dionex ICS-3000 system (Sunnyvale, CA, USA) equipped with suppressed conductivity detection. Anions were separated via an IonPac AS11-HC ion-exchange column (250 x 4 mm) with a potassium hydroxide gradient eluent (flow rate 1.5 ml/min).

2.5. Soil chemical properties

Soil chemical properties were assessed at the end of the experiment (i.e. after 360 days of cultivation) by the "Laboratory of physical and chemical soil analysis" of University of Naples "Federico II" (Department of Agricultural Sciences, Portici). Briefly, soil pH, electrical conductivity (EC), organic matter (OM) and organic carbon (OC) content, total nitrogen (total N), total and active carbonates (limestone), cation exchange capacity (CEC), available phosphate (P_2O_5) and exchangeable bases (Ca^{2+}, Mg^{2+}, K^+, Na^+) were estimated by the standard methods of Sparks (1996). Nitrate ($N\text{-}NO_3^-$) and ammonium ($N\text{-}NH_4^+$) concentrations were assayed with Hach-Lange DR 3900 spectrophotometer equipped with standard vial test: LCK 340 (5-35 mg l^{-1} $N\text{-}NO_3^-$) and LCK 303 (2-47 mg l^{-1} $N\text{-}NH_4^+$).

2.6. Soil microbiological analyses

To study the effect of soil treatments on soil biological properties, three types of approaches were used. Total microbial activity was measured using fluorescein diacetate (FDA) analysis. This method is based on the hydrolysis of fluorescein diacetate (3' 6' -diacetyl-fluorescein) by soil enzymes like esterases, proteases and lipases, and the consequent quantification of the released fluorescein by spectrophotometer measure (490 nm) (Schnürer and Rosswall 1982).

The functionality of the soil microbial community, also defined as "community-level physiological profile" (CLPP), was assessed using BIOLOG EcoPlates™ (BLG) method. BLG consists in a plate with 96 wells containing 3 sets of 31 different carbon sources plus 1 blank (water) as control, and tetrazolium violet as redox dye. When the carbon source is utilized, the tetrazolium violet is reduced by developing a purple colour. The assay was performed as previously described by Bartelt-Ryser et al. (2005). Briefly, 1 g of sieved soil (mesh 2 mm) was shaken for 30 min in 10 ml of distilled water and allowed to settle for 10 min. Then, 120 µl of the supernatant were diluted 100-fold in a distilled water, mixed, and finally used to inoculate the wells of the BIOLOG EcoPlates™. The plates were incubated at room temperature, and oxidation of carbon sources was measured with a spectrophotometer (Thermomax microtitre plate reader, Molecular Devices, Wokingham, UK) at 590 nm, after 24, 48, 72 and 96 h of incubation. Average well colour development (AWCD) was calculated as the sum of wells with activity per plate, divided by the 31 carbon sources.

Finally, composition and diversity of soil bacterial communities was analysed by Illumina high-throughput sequencing. PowerSoil DNA Isolation kit (Mo Bio Laboratories Inc., Carlsbad, CA) was used to isolate the DNA from 250 mg of soil samples in according to the recommendations of the manufacturer. The V1-V3 regions of the 16S rRNA gene (about 520 bp) was amplified by using primers and PCR conditions reported by Ercolini et al. (2012). Library multiplexing, pooling and sequencing were carried out following the Illumina 16S Metagenomic Sequencing Library Preparation protocol, on a MiSeq platform and using the MiSeq Reagent kit v2, leading to 2x250bp, paired-end reads.

2.7. Bioinformatics and statistical data analysis

Demultiplexed, forward and reverse reads were joined by using FLASH Magoč and Salzberg 2011. Joined reads were quality trimmed (Phred score < 20) and short reads (< 250 bp) were discarded by using Prinseq Schmieder and Edwards 2011. High quality reads were then imported in QIIME (Caporaso et al. 2010). Operational taxonomic units (OTUs) were picked through *de novo* approach and uclust method and taxonomic assignment was obtained by using the RDP classifier and the Greengenes database, following a pipeline previously reported De Filippis et al. (2014). In order to avoid biases due to the different sequencing depth, OTU tables were rarefied to the lowest number of sequences per sample. Weighted and unweighted Unifrac distance matrices and alpha-diversity indices (observed OUT richness, Chao index and Shannon index) were computed by QIIME on rarefied OTU tables. PICRUSt

(Phylogenetic Investigation of Communities by Reconstruction of Unobserved States, http://picrust.github.io/picrust) was used to predict the functional profiles of the samples, as recently reported by De Filippis et al. (2015). Principal Component Analysis (PCA) was carried out on the log transformed abundance table by using dudi.pca function in made4 package. Statistical analyses and plotting were carried out in R environment (https://www.r-project.org).

One-way ANOVA was used to analyse the effect of STs on crop yield and quality, soil chemical and soil microbiological parameters. The relationships between relative changes in crop yield and quality, soil properties including BLG and FDA, and soil microbial community composition were obtained by using Pearson correlation coefficients. Significance levels were calculated at $p < 0.05$ and < 0.01. Two-way ANOVA was used to evaluate the difference in crop production between the first and second experimental year. Statistical analyses were performed by STATISTICA software.

3. Results

3.1. Crop yield

At the end of second experimental year, a different response in crop yield (dry weight) was observed between soils with organic amendments and mineral fertilizers (Fig. 1) The highest yields were observed when alfalfa and glucose were combined and applied in a single dose once a year, independently of the presence of biochar (ST 6 and ST 7) (Fig. 1). The other treatments with organic amendments showed a production comparable with untreated soil (ST 1), as well as with the use of mineral fertilizer (ST 2). Finally, very low crop yield was recorded with soil fumigation (Metham-Na) and applications of mineral fertilizer (ST 3) (Fig. 1).

Comparing the crop yield at the end of the first and second experimental year (Fig. 2), we found that ST and experimental year significantly affected crop yield ($p < 0.01$). An increase in crop production was observed for several STs with organic amendments (ST 11 > ST 6 > ST 7 > ST 5), although only ST 11 showed a significative difference ($p < 0.05$) between the years. Interesting, instead, is the significant reduction ($p < 0.01$) of crop yield of 66.5 % for ST 2 and 77.0 % for ST 3 between the first and second experimental year (Fig. 2).

3.2. Seedling establishment and plant survival

To compare the effects of different soil treatments on seedling establishment and plant survival, five plastic collars were placed on soil surface and 10 seeds were sown in each of them. Then, the number of live plants inside the collars were monitored weekly (Fig. 3). After

7 days from sowing, soil treated with organic amendments showed higher number of plants (an average of 73% of established seedlings) than untreated soil (63.3% of established seedlings), except than ST 8, in which a value of 48% of live plants was recorded. In contrast, very low percentage of seedlings was observed for ST 3 (20%) and ST 2 (10%) after 7 days from sowing (Fig. 3). After two weeks from sowing, ST 5, ST 8 and ST 11 showed a slight decrease in the percentage of live plants, whereas for the remain STs an increase of this parameter was observed, with the greatest percentage of live plants detected for ST 6 (79.3%). Subsequently, all STs showed a more or less marked reduction in the percentage of live plants over time. Finally, at the end of the cultivation cycle (i.e., after 50 days) a percentage of live plants greater than 50% was detected with the follows rank ST 6 > ST 1 > ST 9 > ST 7 > ST 11. STs based on the use of synthetic fertilizers showed a final percentage of live plants of 20.7 % for ST 3 and 12 % for ST 2. The remaining STs showed, at the end of the cycle, a percentage of live plants ranging between 30% and 45% (Fig. 3).

3.3. Nitrate content of leaves

Crop quality was evaluated by measuring the content of nitrate (NO_3^-) in leaves after the harvest. The use of synthetic fertilizers (i.e., ST 2 and ST 3) as well as the addition of alfalfa straw and glucose as single dose (i.e., ST 6 and ST 7) showed higher accumulation of NO_3^- in leaves than other treatments, with value ranging from 58 g kg^{-1} of dry matter for ST 7 to 70 g kg^{-1} of dry matter for ST 6, and intermediate values for ST 2 and ST 3 (Fig. 4). On the contrary, STs with low weekly doses of organic amendments applicated in soil containing biochar (i.e., ST 9 and ST 11) resulted in very low content of NO_3^- in leaves (< 10 g kg^{-1} of dry matter), with value comparable to untreated soil (ST 1) (Fig. 4). The remaining organic STs showed an intermediate content of NO_3^-, with value of ~30 g kg^{-1} of dry matter for ST 4, ST 5 and ST 10, and slightly lower (23 g kg^{-1} of dry matter) for ST 8 (Fig. 4).

3.4. Soil chemical properties

At the end of the second experimental year, soil chemical parameters resulted strongly affected by STs (Table 1). Compared with untreated soil (ST 1), where an alkaline pH (8.43) was recorded, the addition of organic material showed a lower pH with values including in sub-alkaline (ST 6 < ST 7 < ST 4) and alkaline (ST 5 < ST 11 < ST 10 < ST 8 < ST 9) range. On the contrary, the use of synthetic fertilizers strongly reduced pH at value of 6.5 (sub-acidic) for SH 3 and pH=5.21 (acidic) for ST 2. EC dramatically increased with the use of synthetic

fertilizers (ST 2 and ST 3), whereas lower values was recorded in soil treated with organic amendments (ST 4 to ST 11) and untreated (ST 1) (Table 1). OM as well as OC contents were higher for the soils with single application of organic amendments (ST 4 to ST 7) than with the use of synthetic fertilizers (ST 2 and ST 3), and intermediate for the treatments with frequent application of organic amendments (SH 8 to SH 11). Total N was higher for the soils amendment with single application of organic materials than others STs, whereas C/N ratio decrease according to the following order: application/s of manure > application/s of alfalfa > applications of mineral fertilizers (Table 1). Soil fumigation combined with applications of synthetic fertilizers (ST 3) strongly increase the level of N-NH_4^+, whereas very high content of N-NO_3^- was recorded with the use of synthetic fertilizers (ST 2 and ST 3) and application of alfalfa as single dose (ST 6 and ST 7). Compared with the use of organic amendments (ST 4 to ST 11), the application of synthetic fertilizers (ST 2 and ST 3) showed a lower value in K^+, Ca^{2+}, total limestone content and CEC, whereas high value was observed for Mg^{2+} and Na^+ content. Finally, P_2O_5 was higher for the soil treated with single application of organic materials (ST 4 to ST 7) and application of synthetic fertilizers (ST 2) than others STs (Table 1).

3.5. Soil microbiological analyses

Compared with untreated soil (ST 1), the application of organic amendments increased both the level of total microbial activity (FDA) that the functionality of soil microbial community (AWCD) (Table 1). On the contrary the lowest levels of microbial activity were observed for the soil with the addition of synthetic fertilizers (ST 2 and ST 3) (Table 1). In particular, after 96 h of incubation, ST 2 showed a lowest value of specific carbon source utilization for all substrate classes that are included in BIOLOG plate (i.e., amine, amino acids, carbohydrates, carboxylic acids, complex carbon source and phosphate carbon) (Fig. 5).

Composition and diversity of soil bacterial communities analysed by Illumina high-throughput sequencing revealed significant changes in response to different STs (Fig. 6). In particular, STs with use of synthetic fertilizers (ST 2 and ST 3) showed lower diversity and richness of bacterial community than in soil amended with organic materials, especially when the soil was previously fumigated (ST 3). Among the soil treated with organic materials, high bacterial richness and diversity were observed with the application of alfalfa at single dose (ST 6) (Fig. 6).

Considering bacterial composition at phylum level (Fig. 7), application of synthetic fertilizers (ST 2 and ST 3) reduced the abundance of Nitrospirae, Chloroflexi and especially Acidobacteria, compared with others STs. On the contrary, Bacteroidetes and Proteobacteria abundance was highest for ST 3 and ST 2, respectively. Finally, very high abundance in Cyanobacteria was observed for untreated soil (ST 1) and for soils with frequent application of manure (ST 8 and ST 9) (Fig. 7).

Principal component analyses (PCA) based on bacterial composition at phylum level clearly separated the STs with the use of synthetic fertilizers (ST 2 and ST 3) from the others (Fig. 8). Among the organic treatments, soils with application of alfalfa at single dose (ST 6 and ST 7) were separated from the other treatments (Fig. 8).

3.6. Linking crop yield and quality, soil chemical properties and microbiota composition

Correlation analisys between crop yield and quality, soil chemical properties and microbiota composition produced 190 Pearson correlation coefficients (Table 2). Crop yield was positively related with organic C soil content, total N, total limestone, CEC, K^+ and total microbial activity (FDA), whereas significant negative correlation was found with Na^+ soil content. Nitrate in leaves was positively related with EC, total N and soil nitrate content (N-NO_3^-), and negatively with pH and C/N ratio (Table 2). Among soil properties, pH and EC were negatively related between them, and opposite Pearson values were observed with C/N ratio, N-NO_3^- and CEC. Soil organic C content was positively related with total N, but no correlation was found with nitrate and ammonium. In contrast, N-NO_3^- showed strong negative correlation with C/N ratio (Table 2). Finally, considering microbiological properties, both total microbial activity (FDA) that functionality of microbial community (AWCD) showed significant positive correlation with pH and negative with EC. In addition, FDA also positively related with organic C, C/N ratio and some soil mineral nutrients (Table 2).

Pearson correlation values between metagenomic results, crop yield and soil properties are reported in Table 3. The results shown that crop yield positively related with Chlamydiae, Firmicutes, Nitrospirae and microbial diversity (i.e., Shannon index). Acidobacteria, Armatimonadetes, Chloroflexi, Cyanobacteria and Nitrospirae were negatively related with nitrate leaves content, EC, total N, N-NO_3^- and N-NH_4^+ and positively with pH, C/N and CEC (Table 3). On the contrary, an opposite correlation pattern was observed for Bacteroidetes and Proteobacteria. Finally, microbial richness and diversity showed significant positive

correlation with pH, CEC, Ca^{2+} and FDA, and negative correlation with EC, N-NH_4^+ and Mg^{2+} (Table 3).

4. Discussion

At the end of the second experimental year the yield, quality and health of crop, as well as soil chemical properties and soil microbial community were significantly affected by different STs. Organic matter, by interacting with both biotic and abiotic soil components, play a fundamental role for the conservation and restoration of soil fertility (Diacono and Montemurro 2010). Several authors compared the effects of synthetic fertilizers and organic amendments on soil properties and crop production (Bonanomi et al. 2011; Goldstein et al. 2004; Mäder et al. 2002; Melero et al. 2006). Although the effects largely depend by the type of organic amendment, plant species, agricultural practices and environmental conditions, including soil type and growing conditions (i.e., open field or greenhouse) (Ahmad et al. 2016; Shi et al. 2009; Steiner et al. 2007), the application of organic amendment is necessary to integrate the quantity of organic C removed by crop production and maintain a good level of soil fertility (Diacono and Montemurro 2010).

In agreement with several authors, the absence of organic matter input into soil and the exclusive use of synthetic fertilizers to support plant growth negatively affects soil organic C (Ge et al. 2011; Li et al. 2012; Marschner et al. 2003; Ros et al. 2006). In our study, the higher organic C content in soil treated with organic amendments could be attributed to the direct effect of organic material application, while the difference in soil organic C between single and repeated applications are probably due to the different application forms, i.e., powdered organic material for single application compared to liquid extract for weekly application. The intensive use of synthetic fertilizers for a long period, especially under protection condition is often associated to soil acidification (Barak et al. 1997; Shi et al. 2009). In our study, soil pH was acid and sub-acid for mineral treatments (ST 2 and ST 3) and sub-alkaline for both untreated (ST 1) and organic treatments (from ST 4 to ST 11). Decrease in soil pH may be due to several soil processes including nutrient leaching, oxidation of iron and manganese and nitrification process (Bolan and Hedley 2003). Ju et al. (2007) reported that excessive application of N mineral fertilizers strongly decreases soil pH as a consequence of intensive nitrification process. We found that total N in soil amended with single application of organic materials was as high as in soil with synthetic fertilizer. In agreement with Ros et al. (2006), this difference could be associated to a direct effect of organic N derived from organic materials. On the

contrary, nitrate soil content ($N\text{-}NO_3^-$) was extremely high for mineral treatments (ST 2 and ST 3) and for single application of alfalfa and glucose (ST 6 and ST 7). In other word, the direct application of N fertilizers and the easy decomposition of alfalfa may be responsible of the observed results (Bonanomi et al. 2013). In addition, soil fumigation strongly increases ammonium soil content ($N\text{-}NH_4^+$), probably due to at the marked decrease in the number of nitrifying bacteria (Tanaka et al. 2003), as confirmed by the negative correlation between $N\text{-}NH_4^+$ and Nitrospirae abundance. Finally, CEC and levels of mineral nutrients like P_2O_5, K^+ and Ca^{2+} are usually higher in organic than in mineral treatments, in according to several authors (Ge et al. 2011; Liu et al. 2007).

While the benefits derived by the use of organic amendments on soil physical, chemical and biological properties are well documented, the role of organic materials as alternative to synthetic fertilizers to support plant growth is still in debate, as reported by the conflictual results of some studies (Bulluck et al. 2002; Hewlett and Melchett 2008; Järvan and Edesi 2009; Melero et al. 2006). In a recent study, Seufert et al. (2012) used a meta-analysis approachs to examine the yield performance of organic and conventional agricultural systems. Their results showed that crop yields in organic systems were generally lower than in conventional ones, with percentage of reduction ranging from 5% to 34% depending on the plant species, agricultural management practices and site characteristics. However, the adoption of organic agricultural practices can reduce the environmental impact compared to conventional system by reducing input-energy levels, gas emission and pollution (Hewlett and Melchett 2008; Mäder et al. 2002). In our study we found that, compared with results of the first experimental year, crop yield during the second year strongly decrease for mineral (ST 2) and fumigated + mineral (ST 3) soil treatments. On the contrary, organic treatments showed an increase of crop biomass according to the amendment types and application frequency, with the highest crop yield observed when alfalfa and glucose were applied at single dose (ST 6 and ST 7) once a year. During the transition from conventional to organic systems a gradual improvement of soil fertility can be observed over the years, therefore a long time is required to increase crop production in newly converted organic systems (Seufert et al. 2012). This could explain the opposite crop yields, among the organic and mineral treatments, observed during the two experimental years. More specifically, crop biomass reduction in soil treated with synthetic fertilizers (ST 2 and ST 3) can be ascribed to a limited plant growth, and a reduction in seedling establishment and plant survival.

The use of different soil fertilization strategies can affect not only soil properties and crop yield, but also food nutritional quality including content of minerals, vitamins, antioxidants and phenols (Wang et al. 2008; Warman and Havard 1997; Worthington 2001). For leafy vegetables such as lettuce, rocket, chicory and spinach, an important parameter to evaluate food quality and security is the nitrate (NO_3^-) leaves content (Fontes et al. 1997). Among leafy vegetables, rocket is considered as an hyper-accumulator of nitrates, and its content widely with soil nitrate content, growth system, season and environmental conditions (Ferrante et al. 2003). Guadagnin et al. (2005) reported that NO_3^- content in rocket produced by the organic system was lower than in conventional system. We found contradictory results, since NO_3^- leaves content largely varied according to the amendment types and application frequency. In fact, N-NO_3^- content in soil amended with alfalfa and glucose at single dose where as high as the application of synthetic fertilizers. In the presence of biochar a slight but no significant reduction in NO_3^- content was observed, while an increase in soil C/N ratio could be a valid strategy to reduce the excessive NO_3^- absorption.

Application of organic matter is also essential to support soil microbial community that, in turn, is involved in several soil processes like organic matter decomposition, nutrient cycling, soil aggregation, plant growth and suppression of soilborne pathogens (Bonanomi et al. 2016b; Bonilla et al. 2012; Bronick and Lal 2005). Generally, enzymatic activity as well as microbial functional diversity was found to be higher in soil with organic than conventional management (Fließbach et al. 2007; Ge et al. 2011; Mäder et al. 2002). In agreement with these findings, we observed that increase of soil EC and reduction of soil pH, organic C and C/N ratio recorded in soil treated with synthetic fertilizers (ST 2 and ST 3) negatively affect total microbial activity (FDA) and community-level physiological profile (AWCD). In the last decade, high-throughput DNA sequencing was used to explore in more detail the impact of application of organic amendment and synthetic fertilizer on soil microbiota (Bonanomi et al. 2016a; Chaudhry et al. 2012). Lauber et al. (2009) reported that richness and diversity of soil bacteria community were strongly related with soil pH. In agreement with their results, we observed that the low pH value in soil with mineral treatment had negative impact on bacterial richness and diversity. On the contrary, addition of organic amendments, by providing food source for microbial populations, increase their abundance and diversity (Bonanomi et al. 2016a; Chaudhry et al. 2012; Sugiyama et al. 2010), but with different response depending byboth on the amendment type and on application frequency. Acidobacteria, Bacteroidetes and Proteobacteria were the most abundant phyla that strongly changed depending on the soil

treatment. Acidobacteria are considered oligotrophic, i.e., organisms that are able to use recalcitrant pool of C and can live in environments with very low level of nutrients (Fierer et al. 2007). In agreement with some studies (Bonanomi et al. 2016a; Hartmann et al. 2015), Acidobacteria abundance increased in soil amended with organic material due to their capability to metabolize recalcitrant organic substrates (Fierer et al. 2007). In other words, the use of synthetic fertilizers, by increasing $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ soil contents, was found to negatively affect Acidobacteria (Fierer et al. 2012). However, in contrast with results of Lauber et al. (2009), a positive correlation between Acidobacteria abundance and soil pH was detected. Some members of Acidobacteria phylum may also have beneficial effect on plant growth and health, since their presence in rhizosphere of healthy plants has been reported to be higher than in the one of diseased plants (Yin et al. 2013). This evidence can partially explain the difference rate of plant survival between organic and synthetic soil treatments. On the contrary, the highest abundance of Proteobacteria and Bacteroidetes was found in mineral and fumigated + mineral soil treatments, respectively. Bacteroidetes and many members of Proteobacteria are considered copiotrophic, i.e., microorganisms that rapid flourish in environments rich of nutrients (Fierer et al. 2007). Among Proteobacteria, Li et al. (2012) found that classes of *Alphaproteobacteria, Betaproteobacteria* and *Gammaproteobacteria* were more abundant in organic than conventional farming system, whereas the opposite pattern was observed for *Deltaproteobacteria*. Contrarily to these authors, we found that *Gammaproteobacteria* and *Deltaproteobacteria* were the most and least abundant, respectively, in the treatments with synthetic fertilizers (ST 2 and ST 3), whereas the other Proteobacteria classes showed variable response. *Betaproteobacteria* and *Gammaproteobacteria* include microorganisms associated with disease suppression, like members of Pseudomonadaceae, Burkholderiaceae and Xanthomonadales families (Bonilla et al. 2012). However, as reported by Mendes et al. (2011), the phenomenon of soil suppressiveness cannot be ascribed to the exclusive presence of a single bacterial taxon or group, but is most likely governed by the presence of microbial consortia.

5. Conclusions

After two experimental years, crop yield, quality and health, as well as soil chemical properties and bacterial community were strongly affected by different soil treatments. The use of synthetic fertilizers, by negatively affecting soil properties, resulted in the reduction of plant survival and consequently decrease of crop yield. On the other hand, organic treatments showed variable response in according to amendment types and application frequencies. Among these,

satisfactory results in terms of crop production and soil fertility were observed for the single application of the mixture alfalfa and glucose, independently of the presence of biochar. Therefore, our study indicates that application of organic materials could be used in intensive agricultural systems in order to reduce the negative effects derived by the application of synthetic fertilizers and soil fumigants in the long-terms. However further studies are necessary to clarify the efficacy of soil organic amendments as alternative or supplement at synthetic fertilizers to support plant growth. In other words, studies that consider different soil types, target species and combination of organic amendment types and application frequencies are needed.

6. References

Ahmad, A.A., Radovich, T.J.K., Nguyen, H. V, Jensen Uyeda, Arakaki, A., Cadby, J., Paull, R., Sugano, J., Teves, G., 2016. Use of Organic Fertilizers to Enhance Soil Fertility, Plant Growth, and Yield in a Tropical Environment. InTech, Rijeka, p. Ch. 0.

Barak, P., Jobe, B.O., Krueger, A.R., Peterson, L.A., Laird, D.A., 1997. Effects of long-term soil acidification due to nitrogen fertilizer inputs in Wisconsin. *Plant and Soil* 197: 61–69.

Bartelt-Ryser, J., Joshi, J., Schmid, B., Brandl, H., Balser, T., 2005. Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. *Perspectives in Plant Ecology Evolution and Systematics* 7: 27–49.

Bennett, A.J., Bending, G.D., Chandler, D., Hilton, S., Mills, P., 2012. Meeting the demand for crop production: The challenge of yield decline in crops grown in short rotations. *Biological Reviews* 87: 52–71.

Blaise, D., 2006. Yield, boll distribution and fibre quality of hybrid cotton (Gossypium hirsutum L.) as influenced by organic and modern methods of cultivation. *Journal of Agronomy and Crop Science* 192: 248–256.

Bolan, N.S., Hedley, M.J., 2003. Role of Carbon, Nitrogen, and Sulfur Cycles in Soil Acidification, in: Handbook of Soil Acidity.

Bonanomi, G., D'Ascoli, R., Antignani, V., Capodilupo, M., Cozzolino, L., Marzaioli, R., Puopolo, G., Rutigliano, F.A., Scelza, R., Scotti, R., Rao, M.A., Zoina, A., 2011. Assessing soil quality under intensive cultivation and tree orchards in Southern Italy. *Applied Soil Ecology* 47: 184–194.

Bonanomi, G., De Filippis, F., Cesarano, G., La Storia, A., Ercolini, D., Scala, F., 2016a. Organic farming induces changes in soil microbiota that affect agro-ecosystem functions. *Soil Biology and Biochemistry* 103: 327–336.

Bonanomi, G., Gaglione, S.A., Cesarano, G., Sarker, T.C., Pascale, M., Scala, F., Zoina, A., 2016b. Frequent Application of Organic Matter to Agricultural Soil Increases Fungistasis.

Bonanomi, G., Incerti, G., Giannino, F., Mingo, A., Lanzotti, V., Mazzoleni, S., 2013. Litter quality assessed by solid state 13C NMR spectroscopy predicts decay rate better than C/N and Lignin/N ratios. *Soil Biology and Biochemistry* 56: 40–48.

Bonilla, N., Gutiérrez-Barranquero, J.A., De Vicente, A., Cazorla, F.M., 2012. Enhancing soil quality and plant health through suppressive organic amendments. *Diversity* 4: 475–491.

Bronick, C., Lal, R., 2005. Soil structure and management: a review. *Geoderma* 124: 3–22.

Bulluck, L.R., Brosius, M., Evanylo, G.K., Ristaino, J.B., 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. *Applied Soil Ecology* 19: 147–160.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature methods* 7: 335–6.

Cataldi, T.R.I., Margiotta, G., Del Fiore, A., Bufo, S.A., 2003. Ionic content in plant extracts determined by ion chromatography with conductivity detection. *Phytochemical Analysis* 14: 176–183.

Chaudhry, V., Rehman, A., Mishra, A., Chauhan, P.S., Nautiyal, C.S., 2012. Changes in Bacterial Community Structure of Agricultural Land Due to Long-Term Organic and Chemical Amendments. *Microbial Ecology* 64: 450–460.

De Filippis, F., Pellegrini, N., Vannini, L., Jeffery, I.B., La Storia, A., Laghi, L., Serrazanetti, D.I., Di Cagno, R., Ferrocino, I., Lazzi, C., Turroni, S., Cocolin, L., Brigidi, P., Neviani, E., Gobbetti, M., O'Toole, P.W., Ercolini, D., 2015. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut* .

De Filippis, F., Vannini, L., La Storia, A., Laghi, L., Piombino, P., Stellato, G., Serrazanetti, D.I., Gozzi, G., Turroni, S., Ferrocino, I., Lazzi, C., Di Cagno, R., Gobbetti, M., Ercolini, D., 2014. The same microbiota and a potentially discriminant metabolome in the saliva of omnivore, ovo-lacto-vegetarian and vegan individuals. *PLoS ONE* 9.

Diacono, M., Montemurro, F., 2010. Long-term effects of organic amendments on soil fertility. A review. *Agronomy for Sustainable Development* 30: 401–422.

Duong, T.T.T., Baumann, K., Marschner, P., 2009. Frequent addition of wheat straw residues to soil enhances carbon mineralization rate. *Soil Biology and Biochemistry* 41: 1475–1482.

Ercolini, D., De Filippis, F., La Storia, A., Iacono, M., 2012. "Remake" by high-throughput sequencing of the microbiota involved in the production of water Buffalo mozzarella cheese. *Applied and Environmental Microbiology* 78: 8142–8145.

Ferrante, A., Incrocci, L., Maggini, R., Tognoni, F., Serra, G., 2003. Preharvest and Postharvest Strategies for Reducing Nitrate Content in Rocket (Eruca Sativa). *Acta Horticulturae* 153–159.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. *Ecology* 88: 1354–1364.

Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *The ISME Journal* 6: 1007–17.

Fließbach, A., Oberholzer, H.-R., Gunst, L., Mäder, P., 2007. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. *Agriculture, Ecosystems & Environment* 118: 273–284.

Fontes, P.C.R., Pereira, P.R.G., Conde, R.M., 1997. Critical chlorophyll, total nitrogen, and nitrate-nitrogen in leaves associated to maximum lettuce yield. *Journal of Plant Nutrition* 20: 1061–1068.

Ge, T., Nie, S., Wu, J., Shen, J., Xiao, H., Tong, C., Huang, D., Hong, Y., Iwasaki, K., 2011. Chemical properties, microbial biomass, and activity differ between soils of organic and conventional horticultural systems under greenhouse and open field management: A case study. *Journal of Soils and Sediments* 11: 25–36.

Goldstein, W., Barber, W., Carpenter-Boggs, L., Daloren, D., Koopmans, C., 2004. Comparisons of conventional, organic and biodynamic methods. *Michael Fields Agricultural Institute. Available at Web site http://www. michaelfieldsaginst. org/education/comparison. pdf (verified 30 June 2008)*.

Guadagnin, S.G., Rath, S., Reyes, F.G.R., 2005. Evaluation of the nitrate content in leaf vegetables produced through different agricultural systems. *Food Additives and Contaminants* 22: 1203–1208.

Hartmann, M., Frey, B., Mayer, J., Mader, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. *ISME J* 9: 1177–1194.

Hewlett, E., Melchett, P., 2008. Can organic agriculture feed the world? A review of the research. *IFOAM organic world congress, Modena, Italy*.

Järvan, M., Edesi, L., 2009. The effect of cultivation methods on the yield and biological quality of potato. *Agronomy Research* 7: 289–299.

Ju, X.T., Kou, C.L., Christie, P., Dou, Z.X., Zhang, F.S., 2007. Changes in the soil environment from excessive application of fertilizers and manures to two contrasting intensive cropping systems on the North China Plain. *Environmental Pollution* 145: 497–506.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* 75: 5111–5120.

Li, R., Khafipour, E., Krause, D.O., Entz, M.H., de Kievit, T.R., Fernando, W.G.D., 2012. Pyrosequencing Reveals the Influence of Organic and Conventional Farming Systems on Bacterial Communities. *PLoS ONE* 7.

Li, X., Zhang, Y., Ding, C., Jia, Z., He, Z., Zhang, T., Wang, X., 2015. Declined soil suppressiveness to Fusarium oxysporum by rhizosphere microflora of cotton in soil sickness. *Biology and Fertility of Soils* 51: 935–946.

Liu, B., Tu, C., Hu, S., Gumpertz, M., Ristaino, J.B., 2007. Effect of organic, sustainable, and conventional management strategies in grower fields on soil physical, chemical, and biological factors and the incidence of Southern blight. *Applied Soil Ecology* 37: 202–214.

Liu, M., Hu, F., Chen, X., Huang, Q., Jiao, J., Zhang, B., Li, H., 2009. Organic amendments with reduced chemical fertilizer promote soil microbial development and nutrient availability in a subtropical paddy field: The influence of quantity, type and application time of organic amendments. *Applied Soil Ecology* 42: 166–175.

Mäder, P., Fliessbach, A., Dubois, D., Gunst, L., Fried, P., Niggli, U., 2002. Soil fertility and biodiversity in organic farming. *Science* 296: 1694–1697.

Magoč, T., Salzberg, S.L., 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27: 2957–2963.

Maksimovic, I., Ilin, Z., 2012. Effects of Salinity on Vegetable Growth and Nutrients Uptake, in: Lee, T.S. (Ed.), Irrigation Systems and Practices in Challenging Environments. InTech.

Mallory, E.B., Griffin, T.S., 2007. Impacts of Soil Amendment History on Nitrogen Availability from Manure and Fertilizer. *Soil Science Society of America Journal* 71: 964.

Marschner, P., Kandeler, E., Marschner, B., 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biology and Biochemistry* 35: 453–461.

Martínez-Blanco, J., Muñoz, P., Antón, A., Rieradevall, J., 2011. Assessment of tomato Mediterranean production in open-field and standard multi-tunnel greenhouse, with compost or mineral fertilizers, from an agricultural and environmental standpoint. *Journal of Cleaner Production* 19: 985–997.

Melero, S., Porras, J.C.R., Herencia, J.F., Madejon, E., 2006. Chemical and biochemical properties in a silty loam soil under conventional and organic management. *Soil and Tillage Research* 90: 162–170.

Mendes, R., Garbeva, P., Raaijmakers, J.M., 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS microbiology reviews* 37: 634–663.

Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H.M., Piceno,

Y.M., DeSantis, T.Z., Andersen, G.L., Bakker, P.A.H.M., Raaijmakers, J.M., 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332: 1097–1100.

Nett, L., Ruppel, S., Ruehlmann, J., George, E., Fink, M., 2012. Influence of Soil Amendment History on Decomposition of Recently Applied Organic Amendments. *Soil Science Society of America Journal* 76: 1290.

O'donnell, A.G., Seasman, M., Macrae, A., Waite, I., Davies, J.T., 2001. Plants and fertilisers as drivers of change in microbial community structure and function in soils Anthony. *Plant and Soil* 232: 135–145.

Ros, M., Klammer, S., Knapp, B., Aichberger, K., Insam, H., 2006. Long-term effects of compost amendment of soil on functional and structural diversity and microbial activity. *Soil Use and Management* 22: 209–218.

Scarascia-Mugnozza, G., Sica, C., Russo, G., 2012. Plastic Materials in European Agriculture: Actual Use and Perspectives. *Journal of Agricultural Engineering* 42: 15–28.

Schmieder, R., Edwards, R., 2011. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 27: 863–864.

Schnürer, J., Rosswall, T., 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Applied and environmental microbiology* 43: 1256–61.

Seufert, V., Ramankutty, N., Foley, J.A., 2012. Comparing the yields of organic and conventional agriculture. *Nature* 485: 229–232.

Shi, W.M., Yao, J., Yan, F., 2009. Vegetable cultivation under greenhouse conditions leads to rapid accumulation of nutrients, acidification and salinity of soils and groundwater contamination in South-Eastern China. *Nutrient Cycling in Agroecosystems* 83: 73–84.

Sparks, D.L., 1996. Methods of Soil Analysis Part 3—Chemical Methods. *Methods of Soil Analysis Part 3—Chemical Methods* i–xxi.

Stark, C.H., Condron, L.M., O'Callaghan, M., Stewart, A., Di, H.J., 2008. Differences in soil enzyme activities, microbial community structure and short-term nitrogen mineralisation resulting from farm management history and organic matter amendments. *Soil Biology and Biochemistry* 40: 1352–1363.

Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., De MacÊdo, J.L.V., Blum, W.E.H., Zech, W., 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant and Soil* 291: 275–290.

Stockdale, E., Shepherd, M., Fortune, S., Cuttle, S., 2002. Soil fertility in organic farming systems – fundamentally different? *Soil Use and Management* 18: 301–308.

Sugiyama, A., Vivanco, J.M., Jayanty, S.S., Manter, D.K., 2010. Pyrosequencing Assessment of Soil Microbial Communities in Organic and Conventional Potato Farms. *Plant Disease* 94: 1329–1335.

Sun, J., Zhang, Q., Zhou, J., Wei, Q., 2014. Pyrosequencing technology reveals the impact of different manure doses on the bacterial community in apple rhizosphere soil. *Applied Soil Ecology* 78: 28–36.

Tanaka, S., Kobayashi, T., Iwasaki, K., Yamane, S., Maeda, K., Sakurai, K., 2003. Properties and metabolic diversity of microbial communities in soils treated with steam sterilization compared with methyl bromide and chloropicrin fumigations. *Soil Science and Plant Nutrition* 49: 603–610.

Tilman, 2002. Agriculture sustainability and intensive production practices 418.

Wang, S.Y., Chen, C.T., Sciarappa, W., Wang, C.Y., Camp, M.J., 2008. Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. *Journal of Agricultural and Food Chemistry* 56: 5788–5794.

Warman, P.R., Havard, K.A., 1997. Yield, vitamin and mineral contents of organically and conventionally grown carrots and cabbage. *Agriculture, ecosystems & environment* 61: 155–162.

Worthington, V., 2001. Nutritional quality of organic versus conventional fruits, vegetables, and grains. *Journal of alternative and complementary medicine (New York, N.Y.)* 7: 161–173.

Yin, C., Hulbert, S.H., Schroeder, K.L., Mavrodi, O., Mavrodi, D., Dhingra, A., Schillinger, W.F., Paulitz, T.C., 2013. Role of bacterial communities in the natural suppression of Rhizoctonia solani bare patch disease of wheat (Triticum aestivum L.). *Applied and Environmental Microbiology* 79: 7428–7438.

Zhou, X., Wu, F., 2015. Changes in soil chemical characters and enzyme activities during continuous monocropping of cucumber (Cucumis sativus). *Pakistan Journal of Botany* 47: 691–697.

Zoran, I.S., Nikolaos, K., Ljubomir, Š., 2014. Tomato fruit quality from organic and conventional production, in: Organic Agriculture Towards Sustainability. InTech.

Figures and tables

Fig. 1. Cumulated crop biomass of 4 cropping cycle expressed as g of dry mass per m^{-2} $year^{-1}$. Data refer to mean of three replicates ± standard deviation. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively. Different letters indicate statistically significant differences between the treatments (Duncan's test at $p < 0.05$).

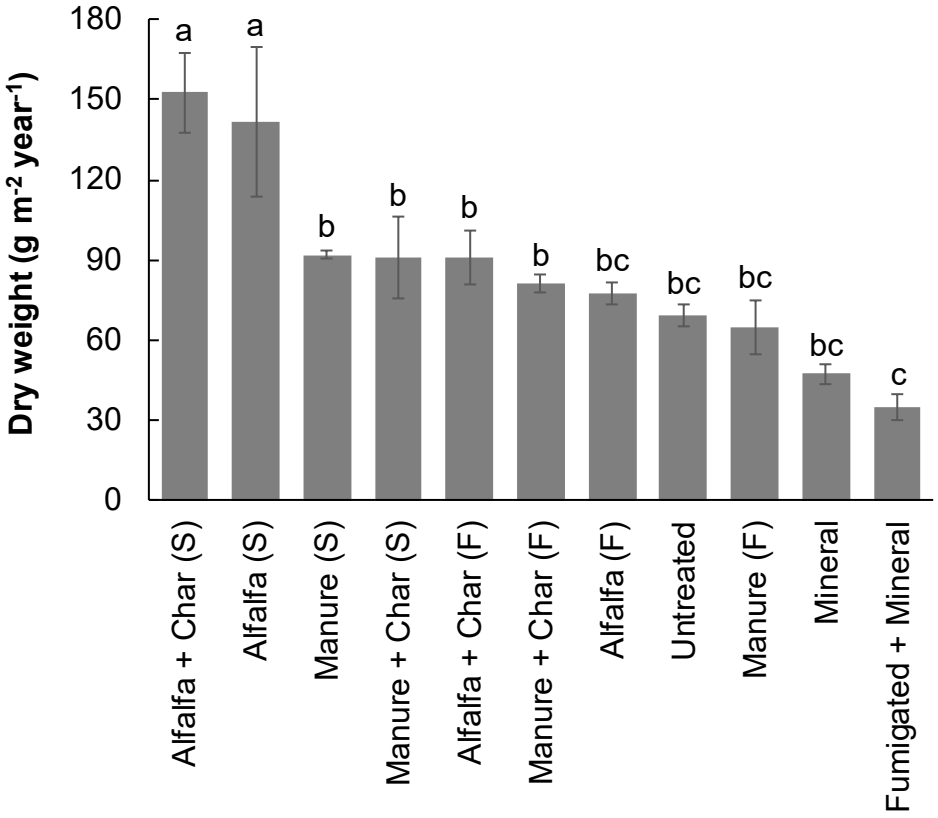

Fig. 2. Variation of cumulated crop biomass (g of dry mass per m^{-2} $year^{-1}$) between first and second year of soil conditioning. Note: the same number of cycles performed during the same months are considered. Asterisks indicate significant statistic differences within each soil treatments by Tukey test (p<0.05). Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

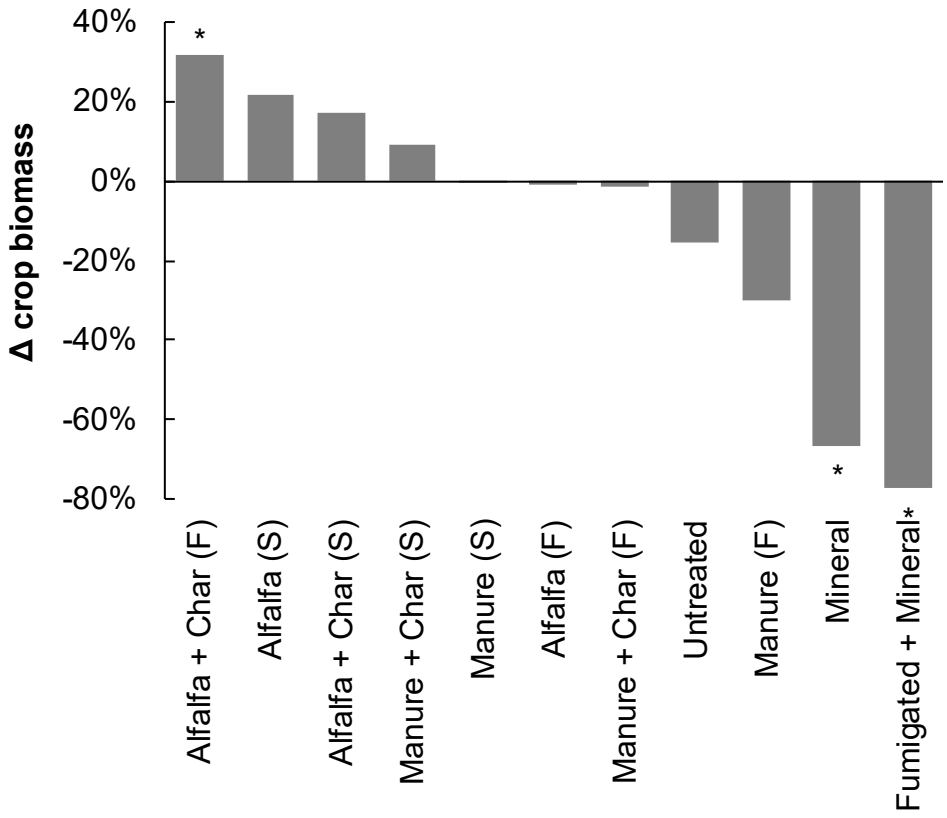

Fig. 3. Percentage of live plants recorded in different treatments during the fourth cultivation cycle. Data refer to mean percentage of all the potentially live plants (i.e. 50 plants). Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

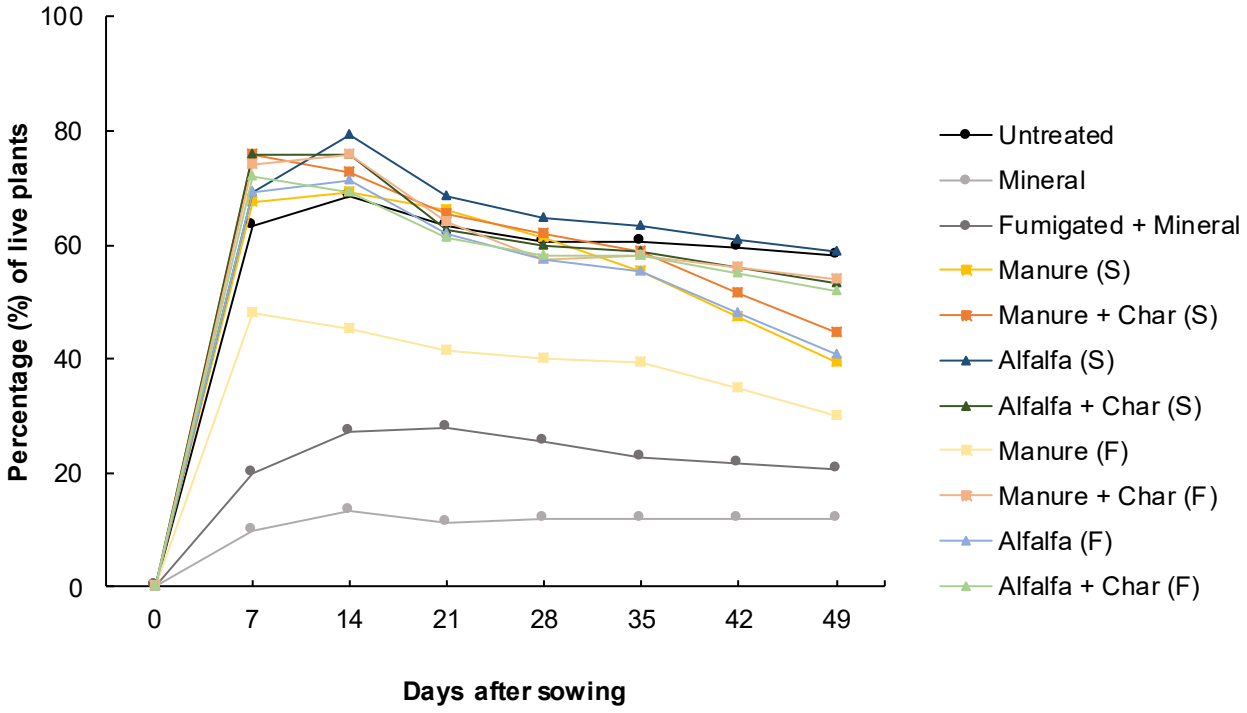

Fig. 4. Nitrate content (g kg^{-1} dry matter) in leaves of rocket grown in mesocosms with different soil treatments. Data refer to mean of four crop cycles ± standard deviation. Different letters indicate statistically significant differences between the treatments (Duncan's test at $p < 0.05$). Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

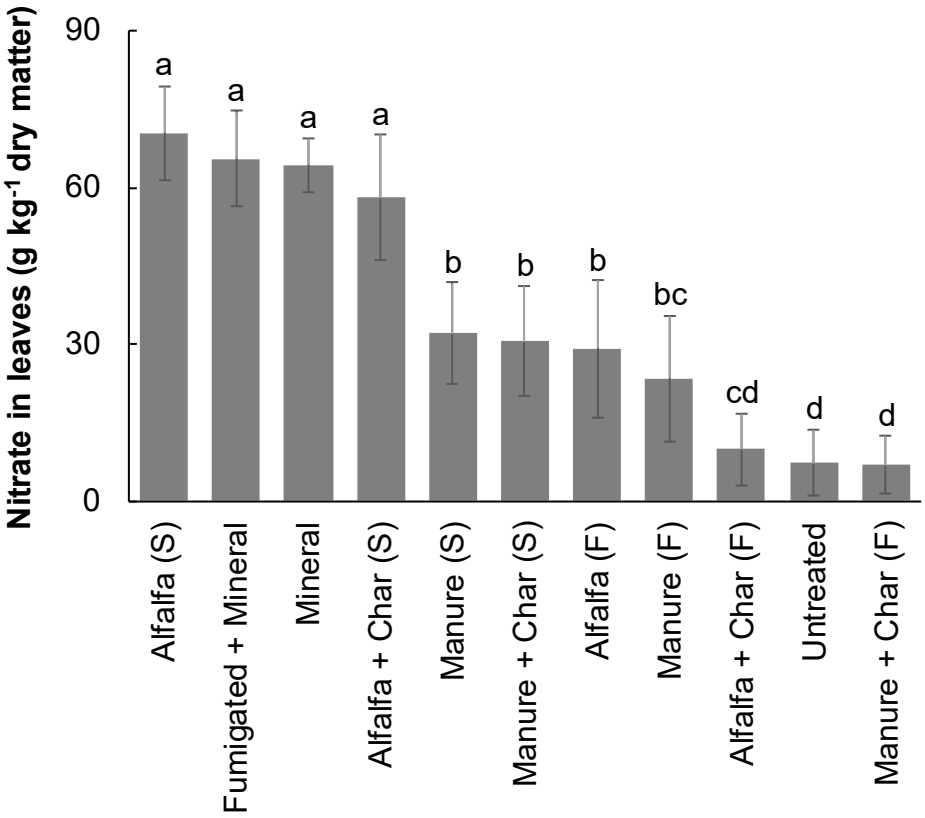

Fig. 5. AWCD recorded for the six main chemical groups in different soil treatments. Absorbance readings at 590 nm after 96 hours of incubation. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

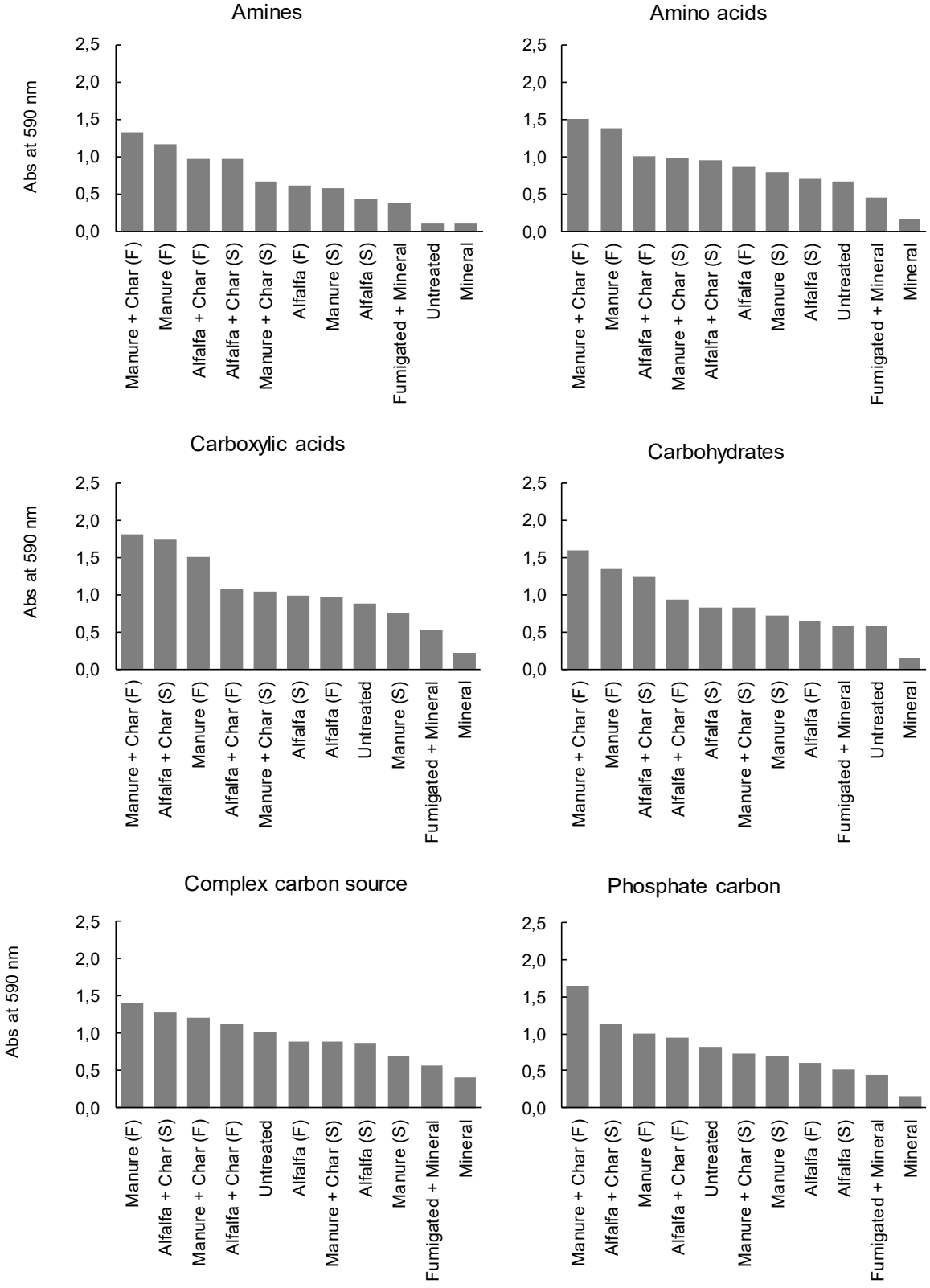

Fig. 6. Box plots showing number of observed OTUs, Chao1 and Shannon diversity indices of bacterial communities in different soil treatments. Boxes represent the interquartile range (IQR) between the first and third quartiles, and the line inside represents the median (2nd quartile). Whiskers denote the lowest and the highest values within 1.5 IQR from the first and third quartiles, respectively. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

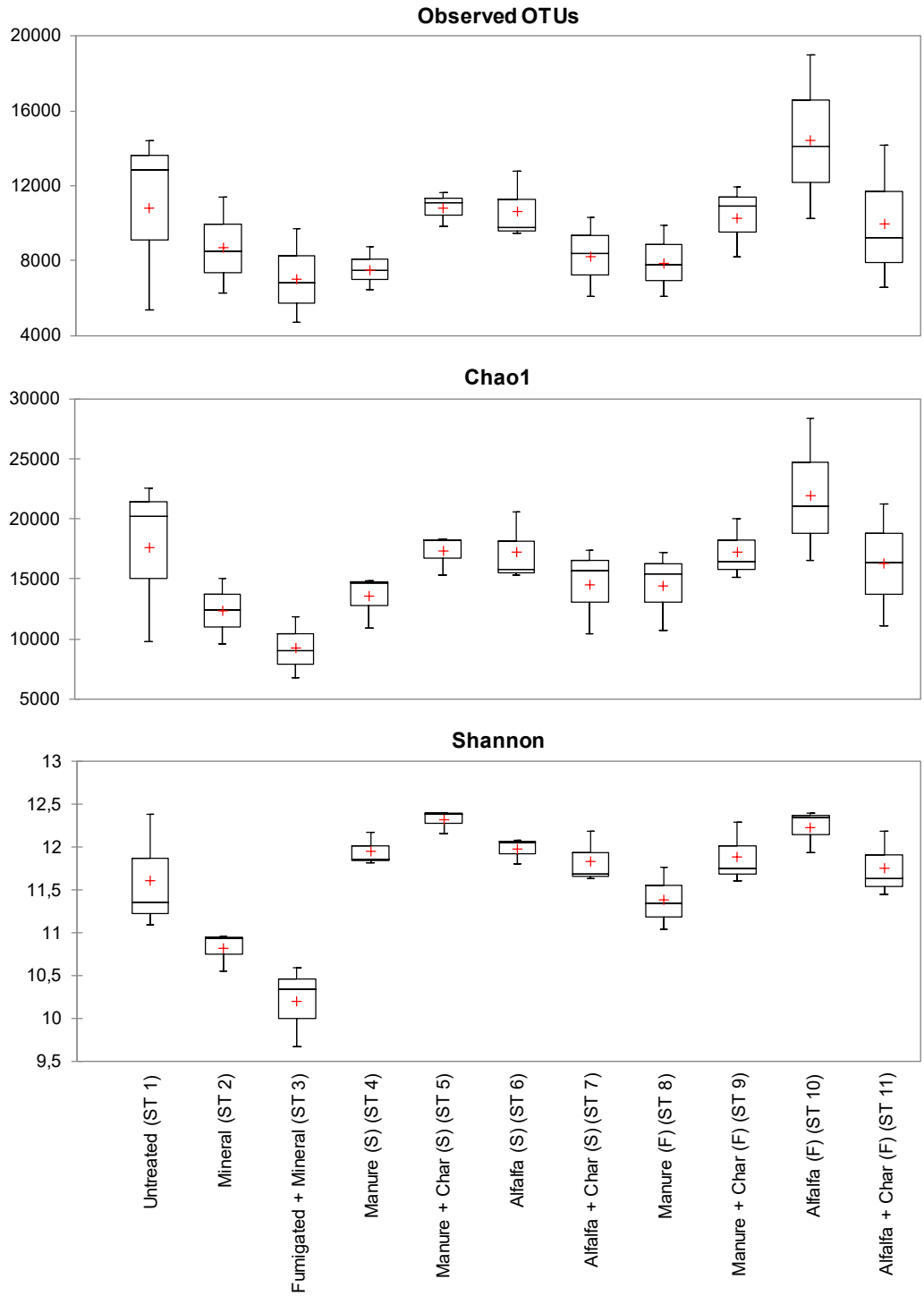

Fig. 7. Bacterial composition (phylum level) of the soil subject to different treatments. The average value of three replicates for each soil treatment is reported. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

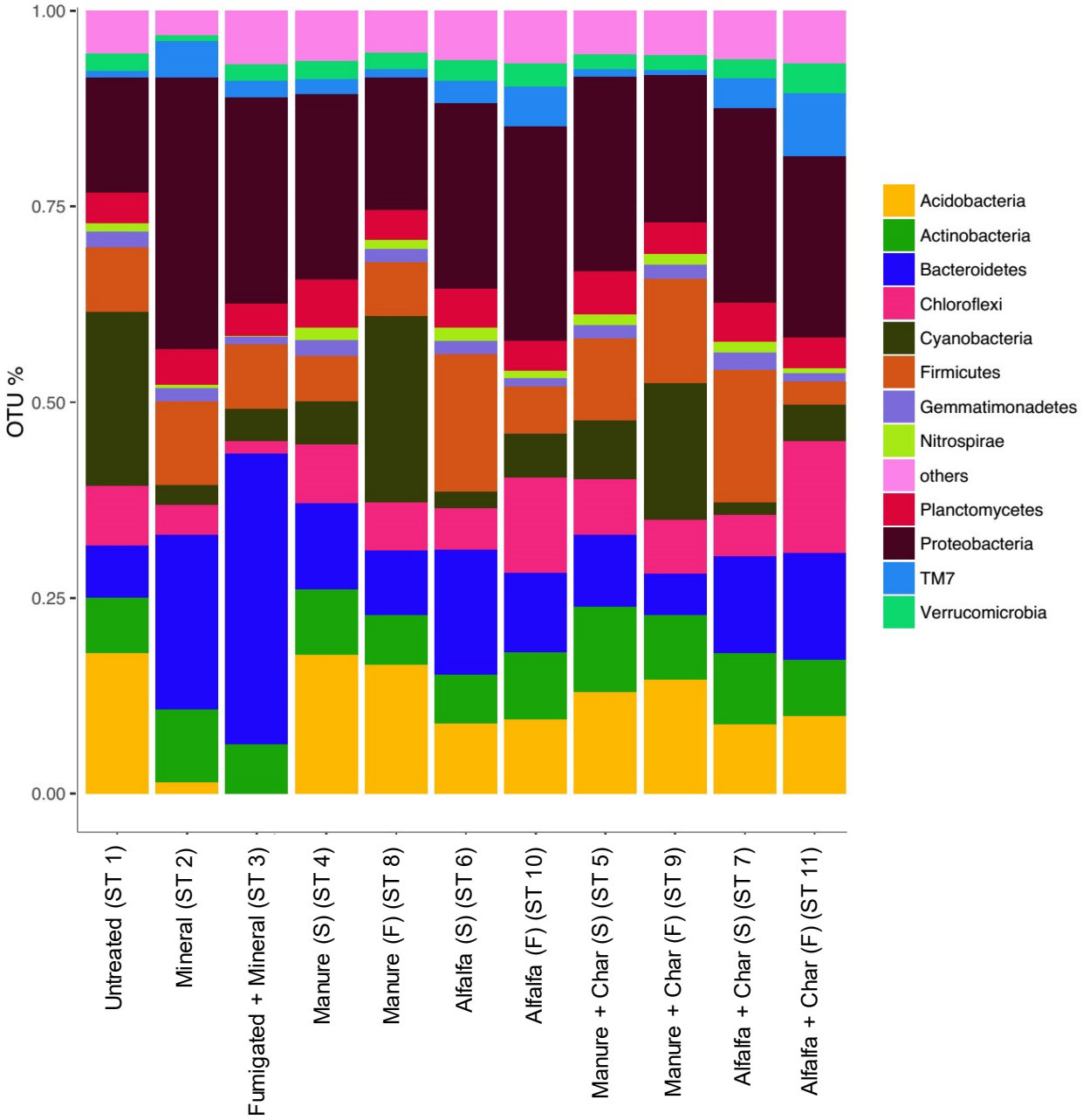

Fig. 8. Principal Component Analysis (PCA) based on the bacterial community composition at genus level. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

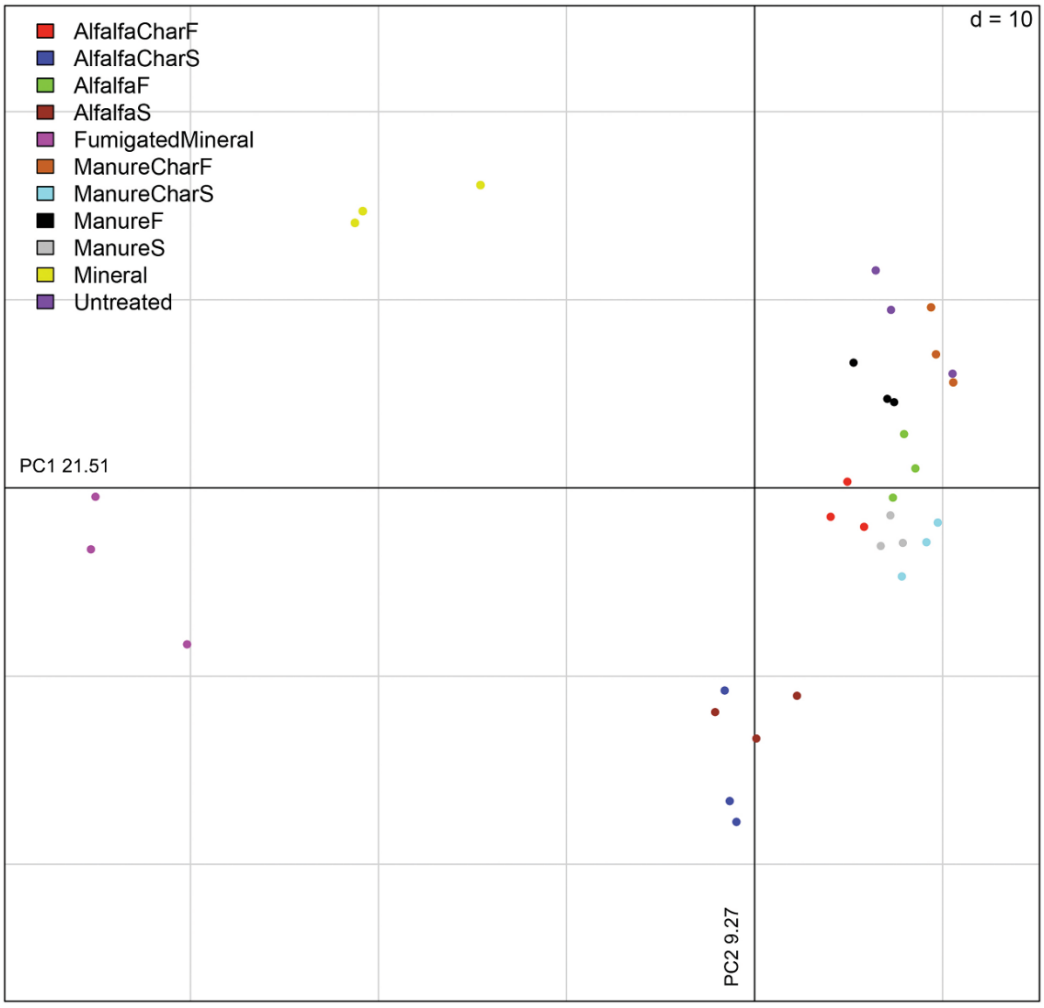

Table 1: Chemical and microbiological parameters under different soil history

	Untreated (SH 1)	Mineral (SH 2)	Fumigated + Mineral (SH 3)	Manure (S) (SH 4)	Manure + Char (S) (SH 5)	Alfalfa (S) (SH 6)	Alfalfa + Char (S) (SH 7)	Manure (F) (SH 8)	Manure + Char (F) (SH 9)	Alfalfa (F) (SH 10)	Alfalfa + Char (F) (SH 11)
Chemical parameters											
pH	8.43 b	5.21 j	6.50 i	8.06 f	8.18 e	7.91 h	8.01 g	8.38 c	8.47 a	8.28 d	8.19 e
EC (µS cm^{-1})	282.50 i	3705.00 a	3545.00 b	481.50 f	429.00 g	762.00 c	655.00 d	387.00 h	293.00 i	523.00 e	515.50 ef
Organic C (g kg^{-1})	14.90 g	14.45 g	14.35 g	26.45 a	25.65 b	23.50 c	21.60 d	17.05 f	17.01 f	17.70 f	18.65 e
Organic matter (g kg^{-1})	25.69 h	24.91 h	24.74 h	45.60 a	44.22 b	40.51 c	37.24 d	29.39 fg	29.08 g	30.51 f	32.15 e
Total N (g kg^{-1})	1.53 e	2.05 c	1.99 c	2.44 b	2.43 b	2.74 a	2.55 b	1.60 e	1.80 d	2.02 c	1.97 c
C/N ratio	9.78 b	7.07 d	7.27 d	10.87 a	10.58 a	8.60 c	8.48 c	10.66 a	9.68 b	8.79 c	9.51 b
N-NO_3^- (mg l^{-1})	4.63 c	134.75 ab	122.00 ab	16.75 c	9.87 c	145.75 a	113.75 b	6.56 c	8.51 c	16.95 c	8.87 c
N-NH_4^+ (mg l^{-1})	0.12 d	5.73 b	50.40 a	0.42 d	0.39 d	1.20 c	0.82 cd	0.14 d	0.14 d	0.69 cd	0.57 cd
Total limestone (g kg^{-1})	8.27 b	2.30 e	1.41 f	9.24 a	7.20 c	8.34 b	7.50 bc	6.94 c	7.24 c	5.47 d	7.23 c
Active limestone (g kg^{-1})	24.85 e	22.65 f	25.30 e	22.40 f	27.35 d	30.05 b	25.20 e	27.70 d	28.70 c	32.45 a	29.60 b
CEC (meq 100g^{-1})	35.60 d	29.35 f	28.70 g	37.00 b	37.65 a	37.20 b	36.55 c	34.95 e	36.56 c	37.25 ab	35.35 de
P_2O_5 (mg kg^{-1})	198.00 i	281.00 c	186.00 j	303.00 a	286.50 b	274.50 d	268.50 e	229.50 f	217.00 g	211.50 h	209.00 h
K^+ (meq 100g^{-1})	1.85 i	2.02 h	1.90 i	2.63 f	3.03 e	4.68 a	4.48 b	2.46 g	2.52 g	3.83 c	3.44 d
Mg^{2+} (meq 100g^{-1})	7.48 bc	8.82 a	8.85 a	6.91 c	8.47 ab	6.76 c	6.94 c	7.45 bc	6.75 c	6.42 c	6.88 c
Ca^{2+} (meq 100g^{-1})	24.69 abc	16.83 d	16.25 d	25.77 a	24.58 abc	24.25 bc	23.79 c	23.34 c	25.27 ab	25.52 ab	23.60 c
Na^+ (meq 100g^{-1})	1.59 b	1.69 a	1.71 a	1.70 a	1.57 b	1.52 c	1.34 e	1.71 a	1.38 e	1.49 c	1.43 d
Microbiological parameters											
FDA (abs 490nm)	0.91 d	0.35 e	0.27 e	1.45 ab	1.57 a	1.54 a	1.21 c	1.40 abc	1.24 bc	1.46 ab	1.21 c
Biolog EcoPlates™ (AWCD)	0.71 e	0.21 g	0.52 f	0.73 e	0.90 d	0.81 de	1.28 b	1.36 b	1.56 a	0.79 e	1.01 c

Means of three replicates ± standard deviations. Different letter within each row indicate significant differences (Duncan test, $p < 0.05$)

Table 2: Cross correlation matrix between crop yield, soil properties and microbiological parameters

	Crop yield	Nitrate leaves	pH	EC	Organic C	Organic matter	Total N	C/N ratio	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$	Total limestone	Active limestone	CEC	P_2O_5	K^+	Mg^{2+}	Ca^{2+}	Na^+	FDA
Nitrate leaves	0.18																		
pH	0.45	-0.67*																	
EC	-0.54	0.66*	-0.96**																
Organic C	0.67*	0.09	0.39	-0.49															
Organic matter	0.67*	0.10	0.39	-0.48	1.00**														
Total N	0.71*	0.62*	-0.07	-0.01	0.78**	0.78**													
C/N ratio	0.17	-0.70*	0.78**	-0.82**	0.52	0.52	-0.11												
$N\text{-}NO_3^-$	0.19	0.94**	-0.70*	0.68*	-0.09	-0.09	0.51	-0.82**											
$N\text{-}NH_4^+$	-0.50	0.46	-0.52	0.72*	-0.40	-0.39	-0.08	-0.57	0.44										
Total limestone	0.66*	-0.48	0.82**	-0.91**	0.64*	0.64*	0.22	0.78**	-0.48	-0.74**									
Active limestone	0.23	-0.25	0.51	-0.44	0.01	0.01	-0.02	0.10	-0.24	-0.21	0.16								
CEC	0.68*	-0.45	0.87**	-0.94**	0.67*	0.67*	0.30	0.71*	-0.52	-0.74**	0.89**	0.46							
P_2O_5	0.46	0.39	-0.16	-0.04	0.75**	0.75**	0.73*	0.18	0.25	-0.42	0.31	-0.38	0.26						
K^+	0.88**	0.28	0.36	-0.41	0.54	0.54	0.70*	-0.02	0.25	-0.38	0.41	0.54	0.59	0.30					
Mg^{2+}	-0.59	0.39	-0.73*	0.76**	-0.30	-0.29	-0.12	-0.39	0.34	0.59	-0.70*	-0.54	-0.75**	0.05	-0.61*				
Ca^{2+}	0.56	-0.60	0.91**	-0.97**	0.56	0.56	0.12	0.77**	-0.64*	-0.75**	0.91**	0.42	0.97**	0.15	0.45	-0.80**			
Na^+	-0.64*	0.24	-0.47	0.49	-0.13	-0.13	-0.20	0.00	0.08	0.40	-0.37	-0.50	-0.53	0.12	-0.64*	0.61*	-0.48		
FDA	0.63*	-0.36	0.81**	-0.88**	0.73*	0.73*	0.34	0.74**	-0.48	-0.70*	0.82**	0.52	0.95**	0.34	0.61*	-0.69*	0.90**	-0.37	
Biolog EcoPlates™	0.41	-0.49	0.73*	-0.69*	0.16	0.16	-0.13	0.53	-0.44	-0.39	0.53	0.41	0.58	-0.15	0.30	-0.56	0.59	-0.59	0.57

Values are Pearson coefficients. Significant differences at the 0.05 (*) and 0.01 (**) are reported. (S) Single, high dose application. (F) Frequent, low dose applications

Table 3: Pearson's correlations between bacterial OTUs collapsed at phylum level, Chao1 and Shannon indices with crop yield and soil properties.

	Acidobacteria	Actinobacteria	Armatimonadetes	Bacteroidetes	Chlamydiae	Chlorobi	Chloroflexi	Cyanobacteria	Firmicutes	Gemmatimonadetes	Nitrospirae	Planctomycetes	Proteobacteria	Verrucomicrobia	Chao1	Shannon
Crop yield	0.23	0.13	-0.16	-0.38	0.74**	-0.42	0.15	-0.33	0.61*	0.46	0.73*	0.45	-0.10	0.39	0.33	0.64*
Nitrate leaves	-0.73*	-0.01	-0.81**	0.70*	0.49	0.54	-0.67*	-0.69*	0.53	0.00	-0.18	0.35	0.66*	-0.32	-0.49	-0.42
pH	0.81**	-0.11	0.67*	-0.77**	0.08	-0.52	0.56	0.46	-0.08	0.18	0.65*	-0.03	-0.77**	0.60*	0.64*	0.75**
EC	-0.86**	-0.04	-0.67*	0.89**	-0.19	0.72*	-0.61*	-0.42	0.03	-0.33	-0.77**	-0.08	0.69*	-0.53	-0.70*	-0.86**
Organic C	0.38	0.37	-0.15	-0.33	0.68*	-0.32	0.14	-0.35	0.21	0.36	0.76**	0.87**	0.01	0.21	0.18	0.67*
Total N	-0.22	0.33	-0.66*	0.14	0.85**	0.02	-0.15	-0.78**	0.53	0.20	0.45	0.79**	0.45	0.09	-0.03	0.34
C/N ratio	0.93**	0.08	0.71*	-0.76**	-0.03	-0.58	0.43	0.55	-0.31	0.34	0.65*	0.27	-0.67*	0.25	0.36	0.63*
$N\text{-}NO_3^-$	-0.78**	-0.12	-0.81**	0.70*	0.44	0.52	-0.66*	-0.63*	0.62*	0.02	-0.24	0.18	0.59	-0.31	-0.49	-0.49
$N\text{-}NH_4^+$	-0.66*	-0.35	-0.50	0.90**	-0.34	0.99**	-0.55	-0.23	-0.09	-0.52	-0.69*	-0.14	0.25	-0.14	-0.67*	-0.80**
Total limestone	0.86**	0.01	0.48	-0.80**	0.38	-0.71*	0.43	0.28	0.11	0.55	0.88**	0.36	-0.63*	0.42	0.50	0.80**
Active limestone	0.06	-0.17	0.33	-0.28	0.22	-0.23	0.56	0.04	-0.03	-0.49	0.09	-0.44	-0.17	0.62*	0.72*	0.47
CEC	0.74**	0.22	0.44	-0.83**	0.46	-0.72*	0.55	0.16	0.14	0.36	0.86**	0.29	-0.46	0.47	0.76**	0.96**
P_2O_5	0.10	0.55	-0.35	-0.16	0.67*	-0.33	-0.20	-0.42	0.37	0.58	0.57	0.87**	0.39	-0.34	-0.09	0.35
K^+	-0.03	0.09	-0.20	-0.21	0.74**	-0.30	0.30	-0.50	0.46	0.05	0.49	0.24	0.13	0.55	0.46	0.60
Mg^{2+}	-0.52	0.18	-0.42	0.63*	-0.34	0.58	-0.62*	-0.09	-0.05	-0.11	-0.60	0.09	0.39	-0.66*	-0.65*	-0.69*
Ca^{2+}	0.84**	0.14	0.57	-0.88**	0.31	-0.74**	0.61*	0.30	0.00	0.35	0.82**	0.18	-0.56	0.48	0.76**	0.92**
Na^+	-0.09	-0.22	0.00	0.42	-0.25	0.40	-0.43	0.14	-0.39	-0.08	-0.33	0.15	0.13	-0.46	-0.53	-0.54
FDA	0.68*	0.16	0.45	-0.75**	0.51	-0.67*	0.52	0.12	0.07	0.25	0.82**	0.32	-0.38	0.47	0.68*	0.91**
Biolog EcoPlates™	0.54	-0.07	0.53	-0.60*	0.00	-0.40	0.23	0.46	0.19	0.26	0.51	-0.17	-0.63*	0.32	0.30	0.45

Values are Pearson coefficients. Significant differences at the 0.05 (*) and 0.01 (**) are reported. (S) Single, high dose application. (F) Frequent, low dose applications

Chapter 5

LONG-TERM ORGANIC MANAGEMENT MODIFIES SOIL MICROBIOTA AND PROMOTES DISEASE SUPPRESSION OF SOILBORNE PHYTOPATHOGENIC FUNGI AND VIRUSES

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Abstract

Applications of soil fumigants and fungicides are widely used in order to control soilborne pathogens. However, long-term application of agrochemicals negatively affects agroecosystem functions, including natural soil disease suppression. To avoid this problem, application of organic amendment is considered a valid strategy. In the present work, long-term effects of organic and conventional managements on soil suppressiveness were compared. In detail, disease suppression in four pathosystems (i.e., *Rhizoctonia solani* – tomato, *Sclerotinia sclerotiorum* – lettuce, *Fusarium oxysporum* f. sp. *raphani* – lettuce and Tomato spotted wilt virus – tomato) was evaluated in soil conditioned for two years with organic and conventional management. Then, the link between disease incidence with soil properties and microbiota was explored. Our results showed that soil amended with organic materials more effectively suppressed *S. sclerotiorum*, *F. oxysporum* f. sp. *raphani* and the infection of TSWV than soil treated with synthetic fertilizers. On the contrary, the incidence of *R. solani* infection was lower in soil treated with synthetic fertilizers than in soil amended with organic materials. Among soil properties, several parameters like pH, EC, C/N, N forms (i.e., N-NO_3^- and N-NH_4^+), FDA and Biolog were differently related with disease incidence depending on the pathosystem. Considering soil microbiota, bacterial richness and diversity, as well as the presence of some genera like Acidobacteria, Chloracidobacteria, Solibacteres Anaerolineae, Nitrospira and Deltaproteobacteria were negatively related with disease incidence in *F. oxysporum* f. sp. *raphani* – lettuce and TWSV – tomato pathosystems, whereas damping off caused by *R. solalni* were negatively affected by the presence of Sphingobacteria and Gammaproteobacteria. Long-term application of organic amendments can effectively improve soil suppressiveness and reduce disease incidence against root and foliar plant pathogens, although the effects varied depending on the pathosystem.

Key Words: Organic amendment; Disease suppression; *Rhizoctonia solani; Sclerotinia sclerotiorum*; *Fusarium oxysporum* f. sp. *raphani;* Tomato spotted wilt virus.

1. Introduction

Within agro-ecosystems, soilborne plant pathogens represent a serious problem to farmers since in the presence of optimal growth conditions they can rapidly spread and compromise the quality and quantity of crop production (Abawi and Widmer 2000). To control soilborne disease, soil fumigants and fungicides have been widely used over the years. However, the long-term use of agrochemicals and other conventional agricultural practices such as monoculture, short rotation and application of synthetic fertilizers, negatively affected agro-ecosystem functions (Giller et al. 1996). At a local scale the extensive use of fungicides reduce soil microbial abundance and diversity (Dungan et al. 2003), and lead to a loss of natural soil suppression (Li et al. 2015), while globally has led to an increase in the number of plant diseases (Fisher et al. 2012), pathogen resistances (Tilman et al. 2002) and environmental pollution (López et al. 2012). To limit the negative effects on the environment, the use of some fumigants, like methyl bromide, has been banned from Europe and other areas of the world, whereas a strong restriction was adopted for many other agrochemicals (Martin 2003). Therefore, to sustain plants health and reduce environmental pollution, ecofriendly management of soilborne pathogens should be adopted.

In organic agricultural systems, several eco-compatible practices are used including crop rotation, soil solarization, biofumigation, application of natural compounds, biocontrol agents and organic amendments (George 2013). Among these, the use of organic amendment (e.g., compost, green manure and animal manure) has been studied for its capability to improve soil fertility (Bulluck et al. 2002), and to control soilborne pathogens (Garbeva et al. 2011). In fact, many studies reported that applications of organic amendment, have positive effects on soil fungistasis and consequently on crop health (Janvier et al. 2007), by increasing enzymatic activities, microbial biomass, function, diversity (Hartmann et al. 2015; Hiddink et al. 2005) and the abundance of beneficial microorganisms (Bonanomi et al. 2016). The main mechanisms involved in the suppression of soilborne pathogens include the competition for nutrients and niches (Lockwood 1990), antibiosis and hyperparasitism (Bonilla et al. 2012). In addition, beneficial microbes can induce systemic resistance in the host plant and reduce the development of airborne disease by interacting with the plant root system (Choudhary et al. 2007). Several studies evaluated the relationship between soil management and the reduction of airborne diseases caused by fungi and bacteria (Tamm et al. 2010; Vallad et al. 2003; Zhang et al. 1996), but no studies addressed the effects on phytopathogenic viruses.

The application of organic materials, however, not always leads to positive effects. Contrasting results are often reported on the application of organic amendment and the induction of soil soppressiveness (as reviewed in Bonanomi et al. 2007). In a study to evaluate the suppressiveness of

a wide range of composts on different pathosystems, Termorshuizen et al. (2006) found a significant disease suppression in 54% of the cases and a disease enhancement in 3%. Mazzola et al. (2001) found that application of *Brassica napus* seed meals suppressed apple root infection by *Rhizoctonia* spp. and *Pratylenchus penetrans* but, in contrast, increased the incidence of *Pythium* spp.. These results suggest that the variability of soil suppressiveness depends on many factors including the considered pathosystem, the organic amendment, soil properties and the environmental conditions.

Most of the previous studies tried to link soil suppressiveness with quality and quantity of organic amendment (Bonanomi et al. 2010; Boulter et al. 2002; Pankhurst et al. 2005) and/or with soil physical, chemical and microbiological parameters (Liu et al. 2007; Tamm et al. 2010; van Bruggen et al. 2015), but few informations are available about the role that the whole soil microbiota have in disease suppression. In our previous study (Chapter 4), the long-term effects of conventional (i.e., soil fumigation and application of synthetic fertilizers) and organic (i.e., different organic amendment types and application frequency) management on soil properties and microbial community composition were evaluated. We found that, compared with conventional system, application of organic amendment significantly improves soil chemical and biological properties, as well as microbial abundance and diversity. However, the effects largely varied according to amendment type and application frequency. Here, we used the previous conditioned soil to investigate the effect of different soil treatments on the induction of soil suppressiveness. Our main objectives were:

i) to test the suppressive capability of 11 soil treatments on four pathosystems including three soilborne pathogens (i.e., *Rhizoctonia solani* – tomato, *Sclerotinia sclerotiorum* – lettuce, *Fusarium oxysporum* f. sp. *raphani* – lettuce) and one virus (i.e., Tomato spotted wilt virus – tomato).

ii) to link changes in soil properties and microbial community composition of different treatments with possible suppressivveness.

2. Material and methods

2.1. Soil samples

Soil used in this experiment it had been previously conditioned for two years with conventional (i.e., soil fumigation and application of synthetic fertilizers) and organic (i.e., different organic amendment type and application frequency) management. Briefly, soil from a farm subjected to intensive cultivation system (i.e., monoculture practice, intensive tillage, application of mineral

fertilizers and soil fumigations with Metham-Na) under plastic tunnel was conditioned with organic and conventional treatments in order to evaluate the effect on crop yield and soil fertility (Chapter 3 and Chapter 4). In detail, a total of 11 soil treatments (STs) were performed as follows: ST 1 - untreated soil (control); ST 2 – soil treated with synthetic fertilizers; ST 3 - soil fumigated by Metham-Na and treated with synthetic fertilizers; ST 4 – soil with a high rate, single application of compost manure at the start of the experiment; ST 5 - soil with a high rate, single application of compost manure plus wood biochar at the start of the experiment; ST 6 – soil with a high rate, single application of glucose and alfalfa straw at the start of the experiment; ST 7 - soil with a high rate, single application of glucose and alfalfa straw plus wood biochar at the start the experiment; ST 8 - soil with low application rates of compost manure added weekly during crop growth; ST 9 - soil with low application rates of compost manure added weekly during crop growth plus wood biochar at the start of the experiment; ST 10 - soil with low application rates of glucose and alfalfa straw added weekly during the whole experiment; ST 11 - soil with low application rates of glucose and alfalfa straw added weekly during the whole experiment plus wood biochar at the start of the experiment (for details see Chapter 3). Organic materials have been chosen considering their different quality and properties. In detail: glucose (N content = 0.00; C/N ratio = ∞) provides a short term labile C for microbes; alfalfa straw (*Medicago sativa*) (N content = 3.93 ± 2.16%; C/N ratio = 11.43 ± 2.98) and compost manure (N content = 3.13 ± 0.64%; C/N ratio = 13.09 ± 1.16) a source of organic N and recalcitrant C; wood biochar (N content = 0.51 ± 0.11%; C/N ratio = 149.61 ± 7.26) provides a safe site for microbial development and promotes soil physical properties (for details about doses and application methods see Chapter 3). Mesocosms (i.e., 32 L plastic tray filled with 35 kg of soil) were set up in triplicate for each STs and placed in greenhouse equipped with automatic control of temperature (24 ± 4°C day and 18 ± 4°C night in spring and summer and 18 ± 4°C day and 12 ± 4°C in fall and winter). During the experiment rocket (*Eruca sativa*) was sown ten times and differences in total crop yield, soil properties and microbial community composition among STs were evaluated at the end of the first (Chapter 3) and second (Chapter 4) experimental year.

At the end of the second experimental year, soil of the three replicas was mixed, air-dried at room temperature and used in plant-pathogen bioassays in order to evaluate the soil suppressiveness of the different STs.

2.2. Soil chemical and microbiological properties

At the end of the second experimental year, soil chemical and microbiological analysis, including total microbial activity, soil microbiome functionality and bacteria community composition were conducted for all STs (see Chapter 4).

Briefly, soil properties including pH, electrical conductivity (EC), organic carbon (OC) content, total nitrogen (total N), total and active carbonates (limestone), cation exchange capacity (CEC), available phosphate (P_2O_5) and exchangeable bases (Ca^{2+}, Mg^{2+}, K^+, Na^+) were evaluated according to standard methods reported by Sparks (1996). Nitrate ($N\text{-}NO_3^-$) and ammonium ($N\text{-}NH_4^+$) soil concentrations were assayed with Hach-Lange DR 3900 spectrophotometer equipped with standard vial test: LCK 340 (5-35 mg l^{-1} $N\text{-}NO_3^-$) and LCK 303 (2-47 mg l^{-1} $N\text{-}NH_4^+$) (see Chapter 4).

Total microbial activity was measured using fluorescein diacetate (FDA) analysis according to method of Schnürer and Rosswall (1982). The functionality of the soil microbial community, also defined as "community-level physiological profile" (CLPP), was assessed using BIOLOG EcoPlates™ (BLG) method, as described by Bartelt-Ryser et al. (2005). Finally, composition and diversity of soil bacterial communities was analysed by Illumina high-throughput sequencing (for details see Chapter 4).

Data of chemical and microbiological analysis of soil obtained at the end of the second experimental year (see results of Chapter 4) were used to explore the effect that STs, had on possible soil suppressiveness by affecting soil chemical and microbiological properties.

2.3. Plant bioassay with soilborne pathogens

Suppressiveness of organic and conventional soil treatments were determined in three pathosystems with soilborne fungal pathogens including: *R. solani* – tomato, *S. sclerotiorum* – lettuce and *F. oxysporum* f. sp. *raphani* – lettuce. Seeds of tomato (cv. Roma) and baby leaf lettuce (cv. Chiara) were purchased from the local market. All bioassay experiments were conducted in greenhouse equipped with automatic control of temperature (22 ± 4°C day and 16 ± 4°C night) and arranged in a completely randomized factorial design.

Inoculum of *R. solani* was produced on a barley medium. Briefly, 150 g of dried barley seeds were placed in 0.5 L flasks, containing potato dextrose broth solution (1/10), autoclaved and, finally, inoculated with plug collected from a colony of *R. solani* grown in a Petri dish. Flasks were incubated for three weeks at 21 °C. At the end, infected seeds were air-dried for one week in sterile conditions and finely grounded. Sterilized pots (16 cm diameter and 20 cm height) were filled with 800 g of air-dried soil of each STs, inoculated with 0.5 % (dry weight) of infected seeds, watered to 80% of field

capacity and incubated in greenhouse. In the control, the same procedure was followed by using non-inoculated barley seeds. Three days after incubation, 15 tomato seeds were sown in each pot. During the experiment, pots were watered every 2-3 days to maintain soil moisture content between 60% and 80% of field capacity. The experimental setup included a total of 11 STs, 2 soil conditions (i.e., soil inoculated with *R. solani* or not) and 8 replicates, for a total of 176 pots. To evaluate soil suppressiveness, the number of healthy plants was monitored weekly until the end of the experiment, i.e., 28 days after sowing. Data recorded at the end were used to calculate damping off index (DOI) as follows:

$$DOI = (HP_{NI} - HP_I) / (HP_{NI}) * 100$$

Where HP_{NI} are the healthy plants in pots without inoculum and HP_I are the healthy plants in pots with inoculum.

Inoculum of *S. sclerotiorum* was produced in the same way as described for *R. solani* (see above). Three days after incubation, 20 lettuce seeds were sown in each pot. During the experiment, pots were watered every 2-3 days to maintain soil moisture content between 60% and 80% of field capacity. The experimental setup included a total of 11 STs, 2 soil conditions (i.e., soil inoculated with *S. sclerotiorum* or not) and 8 replicates, for a total of 176 pots. To evaluate soil suppressiveness, the number of healthy plants was monitored weekly until the end of the experiment, i.e., 42 days after sowing, and damping off index (DOI) was calculated on the last date.

Inoculum of *F. oxysporum* f. sp. *raphani* was produced on potato dextrose agar (PDA) medium. Briefly, Petri dishes containing PDA were inoculated with *F. oxysporum* f. sp. *raphani*. After two weeks, 10 mL of sterile distilled water were added to the Petri dishes and conidia were withdrawn by scraping the culture surface. The spore suspensions were filtered, centrifuged, washed twice with sterile distilled water and adjusted to a concentration of 10^5 conidia mL^{-1} with a hemocytometer. Roots of pre-germinated lettuce seedling were dipped in the conidia suspension and 20 plants were transplanted in each pot (16 cm diameter and 20 cm height) filled with wet soil from different STs. During the experiment, pots were watered every 2-3 days to maintain soil moisture content between 60% and 80% of field capacity. The experimental setup included a total of 11 STs and 8 replicates, for a total of 88 pots. To evaluate soil suppressiveness, the number of healthy plants was monitored weekly for 21 days.

2.4. Plant bioassay with phytopathogenic virus

To evaluate the role that different STs have in the possible induction of plant resistance against airborne pathogens, the TSWV – tomato pathosystem was considered. Bioassay was conducted in a

thermo-conditioned (20–24°C) greenhouse. Sterilized pots (16 cm diameter and 20 cm height) were filled with 800 g of air-dried soil of each STs and watered to 80% of field capacity. One tomato seedling was transplanted in each pot and allow to interact with the soil microbiota for ten days. Subsequently, tomato plants were inoculated with TSWV according to the protocol of Dijkstra and de Jager (2012). Briefly, leaves (1 g) of *Nicotiana glutinosa* infected by TSWV were ground in 10 ml of potassium phosphate buffer (10mM), pH (7-7.2) using a pestle and mortar. Tomato plants were dusted with abrasive carborundum (Fisher Scientific) and cotton swabs dipped in the inoculum were rubbed on cotyledons and early leaves of plants. After inoculation, the plants were sprayed with tap water to remove carborundum from the leaf surface. In the control, the same procedure was performed by using only phosphate buffer as inoculum. During the experiment, pots were watered every 2-3 days to maintain soil moisture content between 60% and 80% of field capacity. The experimental setup included a total of 11 STs, 18 replicates for TSWV and 6 replicates for control, resulting in a total of 264 pots.

Infection symptoms like stunted growth, chlorotic and necrotic rings on leaves, purple veins on the undersides of leaves and plant mortality were recorded periodically after virus inoculation. Finally, three weeks after inoculation, tomato plants were assayed by enzyme-linked immunosorbent assay (ELISA) (Clark and Adams 1977), irrespective of the presence of symptoms. ELISA was carried out by grounding tissues in phosphate buffered saline with Tween 20 (1/10 w/v) and the extracts were tested using a commercial ELISA kit against TSWV (Loewe-Phytodiagnostica Biochemical, Germany). Percentages of infection of inoculated plants were calculated considering the detection of TSWV by ELISA.

2.5. Data analysis

For statistical analysis of the results, data were transformed to satisfy the assumptions of normality and homogeneity of variance, and submitted to analysis of variance (ANOVA) with the software STATISTICA 7. In detail, one-way ANOVA was applied to DOI data to evaluate the significance ($p<0.05$) of different STs on plant protection in presence of soilborne pathogens. Pearson correlation was calculated to assess the link between soil suppressiveness (i.e., DOI for *R. solani* – tomato and *S. sclerotiorum* – lettuce; dead plants for *F. oxysporum* f. sp. *raphani* – lettuce; infected plants and dead plants for TSWV – tomato) and soil chemical and microbiological properties, including total microbial activity, microbial functionality and bacterial community composition. Significance levels were calculated at $p<0.05$ and $p<0.01$.

3. Results

3.1. Suppression of disease caused by soilborne pathogens

In all three pathosystems with soilborne pathogens (i.e., *R. solani* – tomato, *S. sclerotiorum* – lettuce, *F. oxysporum* f. sp. *raphani* – lettuce), STs differently affected soil suppressiveness.

In *R. solani* – tomato pathosystem, DOI index was calculated 28 days after sowing (Fig. 1). Although most STs did not show significant differences, damping off was lower for mineral treatments (ST 2 and ST 3), with a trend to increase with the application of organic materials. Among these last, the lowest DOI was observed for soil treated with single application of alfalfa and glucose (ST 6), whereas the highest value was recorded for ST 9 (i.e., soil with biochar and frequent application of manure) (Fig. 1).

In *S. sclerotiorum* – lettuce pathosystem, the DOI index was calculated 42 days after sowing (Fig. 2). In this case, damping off with organic amendment was generally lower than with synthetic fertilizer. In detail, alfalfa at single dose, with and without presence of biochar (ST 6 and ST 7), showed the lowest DOI value. On the contrary, the highest damping off was recorded for untreated (ST 1) and fumigated + mineral (ST 3) treatments (Fig. 2).

In the *F. oxysporum* f. sp. *raphani* – lettuce pathosystem, the percentage of dead plants was recorded 7, 14 and 21 days after transplanting (Fig. 3). In this case, soil treated with synthetic fertilizers (ST 2 and ST 3) negatively affected plant survival, displaying a percentage of dead plant greater than 35 and 40% for ST 3 and ST 2, respectively, after 21 days of transplanting (Fig. 3). On the contrary, application of organic materials showed a very low percentage of mortality (Fig. 3).

3.2. Suppression of a plant virus

To assess whether different soil treatments could have an effect on plant protection against plant virus, the TSWV – tomato pathosystem was studied. Generally, stunted growth and purple veins on the undersides of leaves were the most common symptoms for all STs, whereas chlorotic and necrotic rings on leaves were less abundant, especially in soil amended with manure (i.e., ST 4 and ST 8) (data not shown). Three weeks after inoculation, the presence of infection in live plants was confirmed by ELISA test. Plants that had died before ELISA test were considered infected. The percentage of infection were high (> 80%) for plants grown in soils treated with synthetic fertilizers (Fig. 4). On the contrary, plants grown on organic soil (i.e., from ST 4 to ST 11) showed a percentage of infection ranging from 5 to 40% of the inoculated plants, depending on amendment type and application frequency. Interesting, more than 60% of infected plants in mineral soil treatments (ST 2 and ST 3)

were dead, while for soil amended with organic materials only a low level of mortality was recorded for ST 6, ST 7 and ST 8 (Fig. 4).

3.3. Linking soil chemical properties with disease suppressiveness

In *R. solani* – lettuce pathosystem, damping off (DOI) showed significant positive correlation with soil pH, C/N ratio, CEC and Ca^{2+} content, whereas negative correlation was found with EC, N-NO_3^- and Mg^{2+}. Positive correlation was also observed with microbial functionality (AWCD) (Table 1). On the contrary, plant mortality in *F. oxysporum* f. sp. *raphani* – lettuce and percentage of infected plants in TSWV – tomato pathosystems were positively related with EC, N-NO_3^-, N-NO_3^- and Mg^{2+}, and negatively related with pH, C/N ratio, CEC, Ca^{2+} and microbiological parameters (i.e., FDA and AWCD) (Table 1). Finally, in *S. sclerotiorum* – lettuce pathosystem, only significant negative correlations between DOI and some soil chemical parameters including organic C, total N, P_2O_5 and K^+ content were found (Table 1).

3.4. Linking soil microbiota with disease suppressiveness

Pearson correlations between soil bacteria and infection bioassays were reported in Table 2. Richness and diversity index of soil bacteria were positively related with *R. solani* DOI and negatively with both plant mortality in *F. oxysporum* f. sp. *raphani* – lettuce and plant infection in TSWV – tomato pathosystems.

Considering the correlation with the 20 most abundant bacterial *taxa,* data analysis showed contrasting results when Pearson values of *R. solani* – tomato pathosystem on the hand, and *F. oxysporum* f. sp. *raphani* – lettuce and TSWV – tomato pathosystems on the other hand are compared (Table 2). In detail, we found that *R. solani* DOI was positively related with several bacterial *taxa*, including members of *Acidobacteria, Chloracidobacteria, Solibacteres, Oscillatoriophycideae* and *Deltaproteobacteria*. On the contrary, significant negative correlations were found with members of *Sphingobacteria* and *Gammaproteobacteria*. Opposite correlations were observed in *F. oxysporum* f. sp. *raphani* – lettuce pathosystem. In addition, plant mortality was also positively affected by *Flavobacteria* and negatively by *Nitrospira* members. Unlike the previous ones, no significant correlation was found between *S. sclerotiorum* DOI and bacterial *taxa* (Table 2).

Finally, we found that mortality in tomato plants infected by TSWV was significantly affected by the abundance of *Sphingobacteria* and *Gammaproteobacteria*, and negatively by the presence of *Acidobacteria* and *Chloracidobacteria* members (Table 2).

4. Discussion

Application of organic amendment is considered as a fundamental practice to sustain soil health and fertility, because of its capability to improve physical, chemical and biological properties of soil (Diacono and Montemurro 2010), including soil disease suppressiveness (Bonilla et al. 2012). In the present study, soil conditioned for two years with conventional (i.e., use of synthetic fertilizers and fumigant) and organic (i.e., use of different organic amendment types and application frequencies) treatments was used to evaluate the effects of different treatments in the induction of soil suppressiveness. In detail, the suppression effects on four pathosystems including three soilborne pathogens (i.e., *R. solani* – tomato, *S. sclerotiorum* – lettuce and *F. oxysporum* f. sp. *raphani* – lettuce) and one virus (i.e., TSWV – tomato) were evaluated. With exception for *R. solani*, the incidence of fungal plant diseases in soil with organic amendments was lower than in conventional treatments. Interestingly, we observed for the first time that long-term use of organic amendments also reduced the incidence of TSWV infection, as well as the mortality of infected plants. However, among organic treatments, the effects largely varied depending on the amendment type and application frequency.

In the last decades, the use of organic amendment as a strategy to increase soil suppressiveness has been extensively studied, although contrasting results have often been reported (Mazzola et al. 2001; Scheuerell et al. 2005; Termorshuizen et al. 2006; Tilston et al. 2002). Some authors reported that suppression efficacy of organic amendments depends on several factors, such as plant pathosystem (Osunlaja 1990), type and quality of organic material (Bonanomi et al. 2013; Pankhurst et al. 2005), and application rate (Boulter et al. 2002). Bonanomi et al. (2017) reported that, compared to non-amended soil, frequent applications of fast decomposing organic material increase soil fungistasis by stimulating the activity of microbial community and, consequently, reduce plant disease. In according with them, we found that application of alfalfa and glucose (i.e., material rich in labile C and organic N) was generally more effective in reducing plant disease than the use of compost manure (i.e, material rich in recalcitrant C compounds). However, we observed contrasting results about dose and application frequency since soil suppressiveness were higher with high single dose of organic amendment than with frequent applications at low dose.

Compared with use of synthetic fertilizers, long-term applications of organic amendments profoundly affected soil physical, chemical and biological properties (see Chapter 4), as confirmed by several studies (Bulluck et al. 2002; Marschner et al. 2003; Melero et al. 2006). On the basis of these results, many authors have tried to relate the changes of both abiotic and biotic factors of soil subjected to organic and conventional management with differences in disease suppressiveness (Liu et al. 2007; Tamm et al. 2010; van Bruggen et al. 2015). Considering chemical properties, some

parameters like soil pH, organic C and N content have been related to disease suppression, although contrasting results are often reported with dfferent pathosystems. For example, Chet and Baker (1980) found that disease incidence of *R. solani* in sugar beet, alfalfa and radish crop was strongly reduced at pH below 6.5, whereas increase in plant disease was observed at pH 8.1. Hoper et al. (1995), instead, reported a positive correlation between soil suppressiveness of *Fusarium* wilt of flax (*F. oxysporum*) and soil pH, whereas negative correlation was found with organic C content. On the contrary, Pankhurst et al. (2002) found that high level of organic C and total N positively affected the suppression of *Gaeumannomyces graminis* var. *tritici* and *R. solani*, while Oyarzun et al. (1998) observed a negative correlation between total N content of soil and its suppressiveness of *Fusarium solani* f.sp. *pisi* on pea. Finally, some authors reported the absence of correlations between soil abiotic parameters and pathogens suppression (see review in Janvier et al. 2007). In our study, we found that correlations between soil properties and disease incidence significantly varied depending on the pathogen. In fact, we found that increase in soil pH was positively related with plant mortality caused by *R. solani* and negatively with *F. oxysporum* f. sp. *raphani* and TSWV, whereas no correlation was observed with *S. sclerotiorum*. Total N and organic C contents were negatively related only with plant mortality in *S. sclerotiorum* – lettuce pathosystem. On the contrary, the forms of N (i.e., $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$) showed significant positive correlations with *F. oxysporum* f. sp. *raphani* and TSWV, and negative correlation with *R. solani*. These results suggest that there is no a universal link between soil chemical properties and soil suppressiveness since different responses can be observed in different pathosystems.

Unlike the soil chemical properties, soil biological parameters like microbial activity and function as well as biomass, diversity and structure of microbial communities, have been reported to be more robust predictors of disease suppressiveness (Bonanomi et al. 2010; Tamm et al. 2010). Several members of soil bacterial community, like *Pseudomonas fluorescens, Bacillus subtilis, Serratia plymuthica, Strepmomyces spp.* and *Lysobacter spp.*, have been found to suppress soilborne pathogens through the adoption of different strategies, including production of antibiotic compounds, parasitism and competition for nutrients and niches (Bonilla et al. 2012; Janvier et al. 2007; Lockwood 1990). For example, Mazzola and Gu (2002) found that antagonistic Pseudomonas strains, effectively suppressed Rhizoctonia root rot in apple tree by producing antibiotic compounds, whereas beneficial strains of *Lysobacter spp.* and *Streptomyces spp.* were found to suppress *R. solani* on sugar beet, *S. scabies* on radish and *Verticillium longisporum* on oilseed rape (Postma et al. 2008). In our experiment, we found a negative correlation between disease incidence in *R. solani* – tomato pathosystem with abundance of Sphingobacteria and Gammaproteobacteria as reported by Mendes

et al. (2011), whereas plants mortality in *F. oxysporum* f. sp. *raphani* – lettuce was negatively affected by several bacterial groups, including members of Acidobacteria, Chloracidobacteria, Solibacteres, Anaerolineae, Nitrospira and Deltaproteobacteria. In other words, the significant correlations that we observed between disease incidence with diversity and richness of soil bacterial community indicate that complexity of the whole microbiota, rather than the exclusive presence or absence of specific taxa, is crucial to soil suppressiveness (Mendes et al. 2011). In this regard, it has been demonstrated that the simultaneous presence of a mixture of soil bacterial isolates (i.e., *Brevundimonas* sp., *Luteibacter* sp., *Pedobacter* sp. and *Pseudomonas* sp.) strongly inhibited the growth of two plant-pathogenic fungi (i.e, *R. solani* and *Fusarium culmorum*) through the production of broad-spectrum antibiotics, while a limited effect was observed when the bacteria were tested individually (De Boer et al. 2007; Garbeva and de Boer 2009).

Finally, some soil microorganisms can indirectly protect the plant against both root and foliar pathogens by inducing a systemic resistance (Choudhary et al. 2007). Bacteria like *Pseudomonas fluorescens*, *Serratia marcescens*, and *Bacillus pumilus* have been found to induce resistance against *Ralstonia solanacearum* (Jetiyanon and Kloepper 2002), *Phytophthora infestans* (Yan et al. 2002), *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Benhamou et al. 1998) and *cucumber mosaic cucumovirus* (Raupach 1996). In a recent study, Tamm et al. (2010) evaluated the impact that soil management and fertilization strategies had on the suppressiveness of soil against soilborne and airborne pathogens. Their results showed that application of organic amendments, by improving soil microbial biomass, had positive effects on suppressiveness against both soilborne and airborne pathogens (Tamm et al. 2010). In our study, the higher richness and diversity of bacteria observed in soil treated with organic amendments than with synthetic fertilizers, negatively affected the incidence of infection in TSWV – tomato pathosystem. Interestingly, plant mortality in plant infected by virus was negatively related with the presence of some bacterial taxa (i.e., members of Acidobacteria, Chloroflexi, Nitrospirae and Deltaproteobacteria) particularly abundant in soil amended with organic materials. In other words, long-term application of organic amendments could promote the presence of soil bacteria involved in the induction of systemic resistance in plants. These results suggest that use of organic amendments could represent a promising strategy for controlling plant viruses. However, more detailed studies are needed to evaluate the robustness of our findings.

5. Conclusion

Within agro-ecosystem, soil health represents an important aspect for crop production and agricultural sustainability. In this regard, application of organic amendment is considered an effective

practice to improve soil properties and natural soil suppressiveness. In our study, long-term application of organic amendments, especially fast decomposing materials, reduced the disease incidence in three pathosystems, including two soilborne pathogens (*Sclerotinia sclerotiorum* and *Fusarium oxysporum* f. sp. *raphani*) and one virus (TSWV), whereas a greater incidence was observed in soil treated with ordinary management. In detail, some soil chemical and biological properties, as well as richness and diversity of soil microbiota were significantly related with disease suppression. However, the higher suppression of *R. solani* in soil treated with ordinary management than with application of organic materials, suggest that a variable response can be observed in different pathosystems. Therefore, further research including a broad range of organic materials and pathosystems is needed in order to generalize the effects that organic amendments have on plant protection.

6. References

Abawi, G.S., Widmer, T.L., 2000. Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. *Applied Soil Ecology* 15: 37–47.

Bartelt-Ryser, J., Joshi, J., Schmid, B., Brandl, H., Balser, T., 2005. Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. *Perspectives in Plant Ecology Evolution and Systematics* 7: 27–49.

Benhamou, N., Kloepper, J.W., Tuzun, S., 1998. Induction of resistance against Fusarium wilt of tomato by combination of chitosan with an endophytic bacterial strain: Ultrastructure and cytochemistry of the host response. *Planta* 204: 153–168.

Bonanomi, G., Antignani, V., Capodilupo, M., Scala, F., 2010. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biology and Biochemistry*.

Bonanomi, G., Antignani, V., Pane, C., Scala, F., 2007. Supression of Soilborne Fungal Diseases with Organic Amendments. *Journal of Plant Pathology* 89: 311–324.

Bonanomi, G., De Filippis, F., Cesarano, G., La Storia, A., Ercolini, D., Scala, F., 2016. Organic farming induces changes in soil microbiota that affect agro-ecosystem functions. *Soil Biology and Biochemistry* 103: 327–336.

Bonanomi, G., Gaglione, S.A., Cesarano, G., Sarker, T.C., Pascale, M., Scala, F., Zoina, A., 2017. Frequent Applications of Organic Matter to Agricultural Soil Increase Fungistasis. *Pedosphere* 27: 86–95.

Bonanomi, G., Gaglione, S. a., Incerti, G., Zoina, A., 2013. Biochemical quality of organic amendments affects soil fungistasis. *Applied Soil Ecology* 72: 135–142.

Bonilla, N., Gutiérrez-Barranquero, J.A., De Vicente, A., Cazorla, F.M., 2012. Enhancing soil quality and plant health through suppressive organic amendments. *Diversity* 4: 475–491.

Boulter, J.I., Boland, G.J., Trevors, J.T., 2002. Assessment of compost for suppression of Fusarium Patch (Microdochium nivale) and Typhula Blight (Typhula ishikariensis) snow molds of turfgrass. *Biological control* 25: 162–172.

Bulluck, L.R., Brosius, M., Evanylo, G.K., Ristaino, J.B., 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. *Applied Soil Ecology* 19: 147–160.

Chet, I., Baker, R., 1980. Induction of suppressiveness to rhizoctonia-solani in soil. *Phytopathology* 70: 994–998.

Choudhary, D.K., Prakash, A., Johri, B.N., 2007. Induced systemic resistance (ISR) in plants: Mechanism of action. *Indian Journal of Microbiology* 47: 289–297.

Clark, M.F., Adams, A.N., 1977. Characteristics of the Microplate Method of Enzyme-Linked Immunosorbent Assay for the Detection of Plant Viruses. *Journal of General Virology* 34: 475–483.

De Boer, W., Wagenaar, A.M., Klein Gunnewiek, P.J.A., Van Veen, J.A., 2007. In vitro suppression of fungi caused by combinations of apparently non-antagonistic soil bacteria. *FEMS Microbiology Ecology* 59: 177–185.

Diacono, M., Montemurro, F., 2010. Long-term effects of organic amendments on soil fertility. A review. *Agronomy for Sustainable Development* 30: 401–422.

Dijkstra, J., de Jager, C., 2012. Practical plant virology: protocols and exercises. Springer Science & Business Media.

Dungan, R.S., Ibekwe, A.M., Yates, S.R., 2003. Effect of propargyl bromide and 1,3-dichloropropene on microbial communities in an organically amended soil. *FEMS Microbiology Ecology* 43: 75–87.

Fisher, M.C., Henk, D. a, Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L., Gurr, S.J., 2012. Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484: 186–94.

Garbeva, P., de Boer, W., 2009. Inter-specific interactions between carbon-limited soil bacteria affect behavior and gene expression. *Microbial Ecology* 58: 36–46.

Garbeva, P., Hol, W.H.G., Termorshuizen, A.J., Kowalchuk, G.A., De Boer, W., 2011. Fungistasis and general soil biostasis - A new synthesis. *Soil Biology and Biochemistry* 43: 469–477.

George, A., 2013. Plant Pathology, Journal of Chemical Information and Modeling.

Giller, K.E., Beare, M.H., Lavelle, P., Izac, a N., Swift, M.J., 1996. Agricultural intensification , soil biodiversity and agroecosystem function. *Applied Soil Ecology* 6: 3–16.

Hartmann, M., Frey, B., Mayer, J., Mader, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. *ISME J* 9: 1177–1194.

Hiddink, G.A., Van Bruggen, A.H.C., Termorshuizen, A.J., Raaijmakers, J.M., Semenov, A. V., 2005. Effect of organic management of soils on suppressiveness to Gaeumannomyces graminis var. tritici and its antagonist, Pseudomonas fluorescens. *European Journal of Plant Pathology* 113: 417–435.

Hoper, H., Steinberg, C., Alabouvette, C., 1995. Involvement of clay type and pH in the suppressiveness of soil to plant diseases. *European Journal of Soil Biology* 32: 41–58.

Janvier, C., Villeneuve, F., Alabouvette, C., Edel-Hermann, V., Mateille, T., Steinberg, C., 2007. Soil health through soil disease suppression: which strategy from descriptors to indicators? *Soil Biology and Biochemistry* 39: 1–23.

Jetiyanon, K., Kloepper, J.W., 2002. Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biological Control* 24: 285–291.

Li, X., Zhang, Y., Ding, C., Jia, Z., He, Z., Zhang, T., Wang, X., 2015. Declined soil suppressiveness to Fusarium oxysporum by rhizosphere microflora of cotton in soil sickness. *Biology and Fertility of Soils* 51: 935–946.

Liu, B., Tu, C., Hu, S., Gumpertz, M., Ristaino, J.B., 2007. Effect of organic, sustainable, and conventional management strategies in grower fields on soil physical, chemical, and biological factors and the incidence of Southern blight. *Applied Soil Ecology* 37: 202–214.

Lockwood, J.L., 1990. Relation of energy stress to behaviour of soil-borne plant pathogens and to disease development [International Congress of Plant Pathology].

López, H.E.F., Orozco, C.D.L.M., Durán, A.A.C., Corral, J.A.R., Vega, H.R., FuentesHernández, V.O., 2012. Nonpoint Pollution Caused by the Agriculture and Livestock Activities on Surface Water in the Highlands of Jalisco, Mexico, in: Abrol, V., Sharma, P. (Eds.), Resource Management for Sustainable Agriculture. InTech, Rijeka.

Marschner, P., Kandeler, E., Marschner, B., 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biology and Biochemistry* 35: 453–461.

Martin, F.N., 2003. Development of alternative strategies for management of soilborne pathogens currently controlled with methyl bromide. *Annual Review of Phytopathology* 41: 325–350.

Mazzola, M., Granatstein, D.M., Elfving, D.C., Mullinix, K., 2001. Suppression of specific apple root pathogens by Brassica napus seed meal amendment regardless of glucosinolate content.

Phytopathology 91: 673–679.

Mazzola, M., Gu, Y.-H., 2002. Wheat Genotype-Specific Induction of Soil Microbial Communities Suppressive to Disease Incited by Rhizoctonia solani Anastomosis Group (AG)-5 and AG-8. *Phytopathology* 92: 1300–1307.

Melero, S., Porras, J.C.R., Herencia, J.F., Madejon, E., 2006. Chemical and biochemical properties in a silty loam soil under conventional and organic management. *Soil and Tillage Research* 90: 162–170.

Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H.M., Piceno, Y.M., DeSantis, T.Z., Andersen, G.L., Bakker, P.A.H.M., Raaijmakers, J.M., 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332: 1097–1100.

Osunlaja, S.O., 1990. Effect of organic soil amendments on the incidence of stalk rot of maize. *Plant and Soil* 127: 237–241.

Oyarzun, P.J., Gerlagh, M., Zadoks, J.C., 1998. Factors associated with soil receptivity to some fungal root rot pathogens of peas. *Applied Soil Ecology* 10: 151–169.

Pankhurst, C.E., Blair, B.L., Magarey, R.C., Stirling, G.R., Garside, A.L., 2005. Effects of biocides and rotation breaks on soil organisms associated with the poor early growth of sugarcane in continuous monoculture. *Plant and Soil* 268: 255–269.

Pankhurst, C.E., McDonald, H.J., Hawke, B.G., Kirkby, C.A., 2002. Effect of tillage and stubble management on chemical and microbiological properties and the development of suppression towards cereal root disease in soils from two sites in NSW, Australia. *Soil Biology and Biochemistry* 34: 833–840.

Postma, J., Schilder, M.T., Bloem, J., van Leeuwen-Haagsma, W.K., 2008. Soil suppressiveness and functional diversity of the soil microflora in organic farming systems. *Soil Biology and Biochemistry* 40: 2394–2406.

Raupach, G.S., 1996. Induced Systemic Resistance in Cucumber and Tomato Against Cucumber Mosaic Cucumovirus Using Plant Growth-Promoting Rhizobacteria (PGPR). *Plant Disease* 80: 891.

Scheuerell, S.J., Sullivan, D.M., Mahaffee, W.F., 2005. Suppression of Seedling Damping-Off Caused by Pythium ultimum, P. irregulare, and Rhizoctonia solani in Container Media Amended with a Diverse Range of Pacific Northwest Compost Sources. *Phytopathology* 95: 306–315.

Schnürer, J., Rosswall, T., 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Applied and environmental microbiology* 43: 1256–61.

Sparks, D.L., 1996. Methods of Soil Analysis Part 3—Chemical Methods. *Methods of Soil Analysis Part 3—Chemical Methods* i–xxi.

Tamm, L., Thürig, B., Bruns, C., Fuchs, J.G., Köpke, U., Laustela, M., Leifert, C., Mahlberg, N., Nietlispach, B., Schmidt, C., Weber, F., Fließbach, A., 2010. Soil type, management history, and soil amendments influence the development of soil-borne (Rhizoctonia solani, Pythium ultimum) and air-borne (Phytophthora infestans, Hyaloperonospora parasitica) diseases. *European Journal of Plant Pathology* 127: 465–481.

Termorshuizen, A.J., van Rijn, E., van der Gaag, D.J., Alabouvette, C., Chen, Y., Lagerlöf, J., Malandrakis, A.A., Paplomatas, E.J., Rämert, B., Ryckeboer, J., Steinberg, C., Zmora-Nahum, S., 2006. Suppressiveness of 18 composts against 7 pathosystems: Variability in pathogen response. *Soil Biology and Biochemistry* 38: 2461–2477.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. *Nature* 418: 671–7.

Tilston, E.L., Pitt, D., Groenhof, A.C., 2002. Composted recycled organic matter suppresses soil-borne diseases of field crops. *New Phytologist* 154: 731–740.

Vallad, G.E., Cooperband, L., Goodman, R.M., 2003. Plant foliar disease suppression mediated by composted forms of paper mill residuals exhibits molecular features of induced resistance. *Physiological and Molecular Plant Pathology* 63: 65–77.

van Bruggen, A.H.C., Sharma, K., Kaku, E., Karfopoulos, S., Zelenev, V. V., Blok, W.J., 2015. Soil health indicators and Fusarium wilt suppression in organically and conventionally managed greenhouse soils. *Applied Soil Ecology* 86: 192–201.

Yan, Z., Reddy, M.S., Ryu, C.-M., McInroy, J. a, Wilson, M., Kloepper, J.W., 2002. Induced systemic protection against tomato late blight elicited by plant growth-promoting rhizobacteria. *Phytopathology* 92: 1329–1333.

Zhang, W., Dick, W.A., Hoitink, H.A.J., 1996. Compost-Induced Systemic Acquired Resistance in Cucumber to Pythium Root Rot and Anthracnose. *Phytopathology*.

Figures and tables

Fig. 1. *R. solani* – tomato damping off index (DOI) in different soil treatments calculated 28 days after sowing. Values are the means of eight replicas. Different letters indicate statistically significant differences between the treatments (Duncan's test at $p < 0.05$). Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

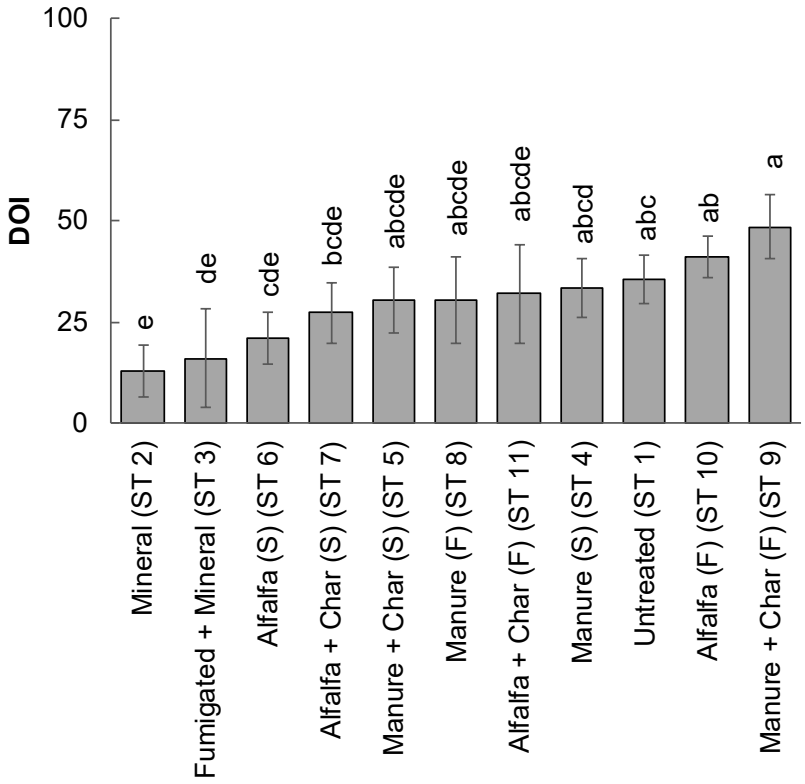

Fig. 2. *S. sclerotiorum* – lettuce damping off index (DOI) in different soil treatments calculated 42 days after sowing. Values are the means of eight replicates. Different letters indicate statistically significant differences between the treatments (Duncan's test at $p < 0.05$). Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

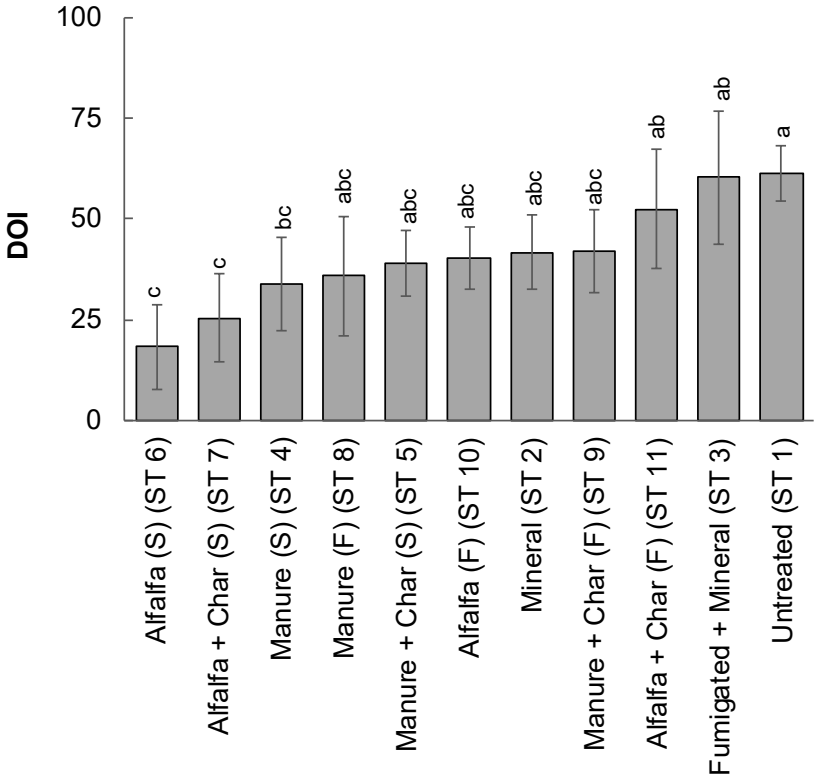

Fig. 3. Percentage of dead plants 7, 14 and 21 days after transplanting in *F. oxysporum* f. sp. *raphani* – lettuce pathosystem. Data refer to mean ± standard deviation (N = 8). Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

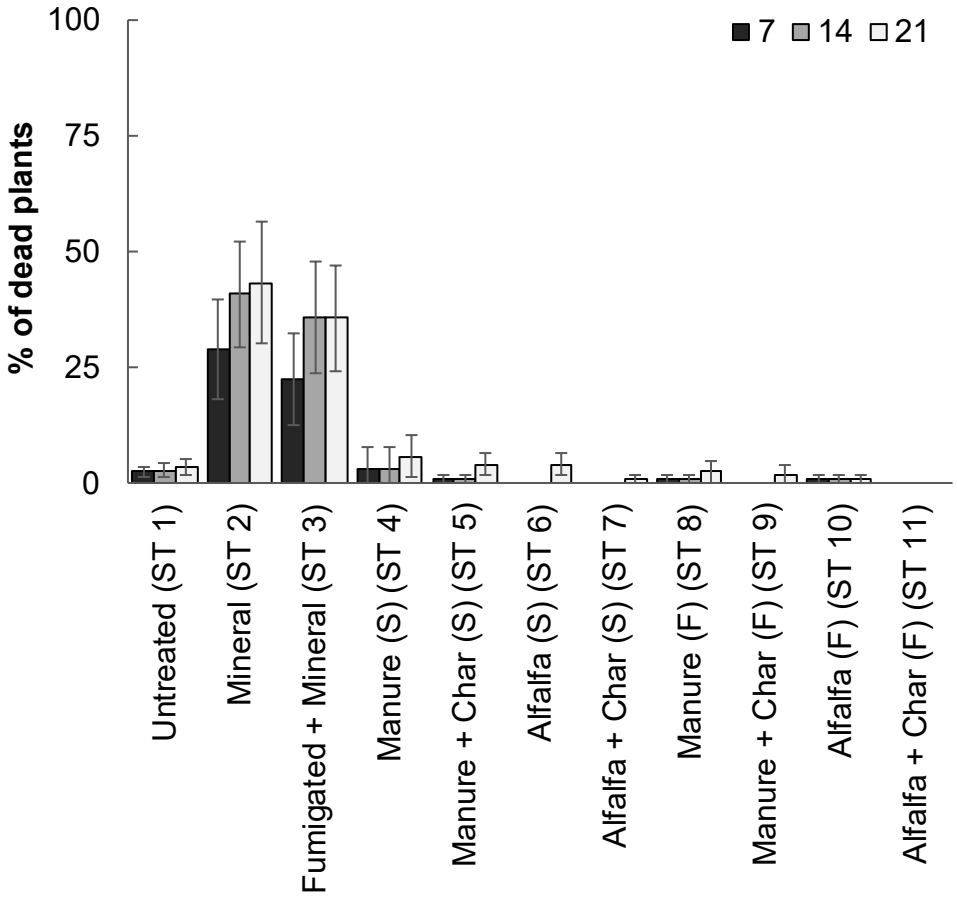

Fig. 4. Percentage of healthy and infected plants in *TSWV* – tomato pathosystem. Values are means of 18 replicas. Application frequency is indicated with (S) and (F) for single and frequent rate, respectively.

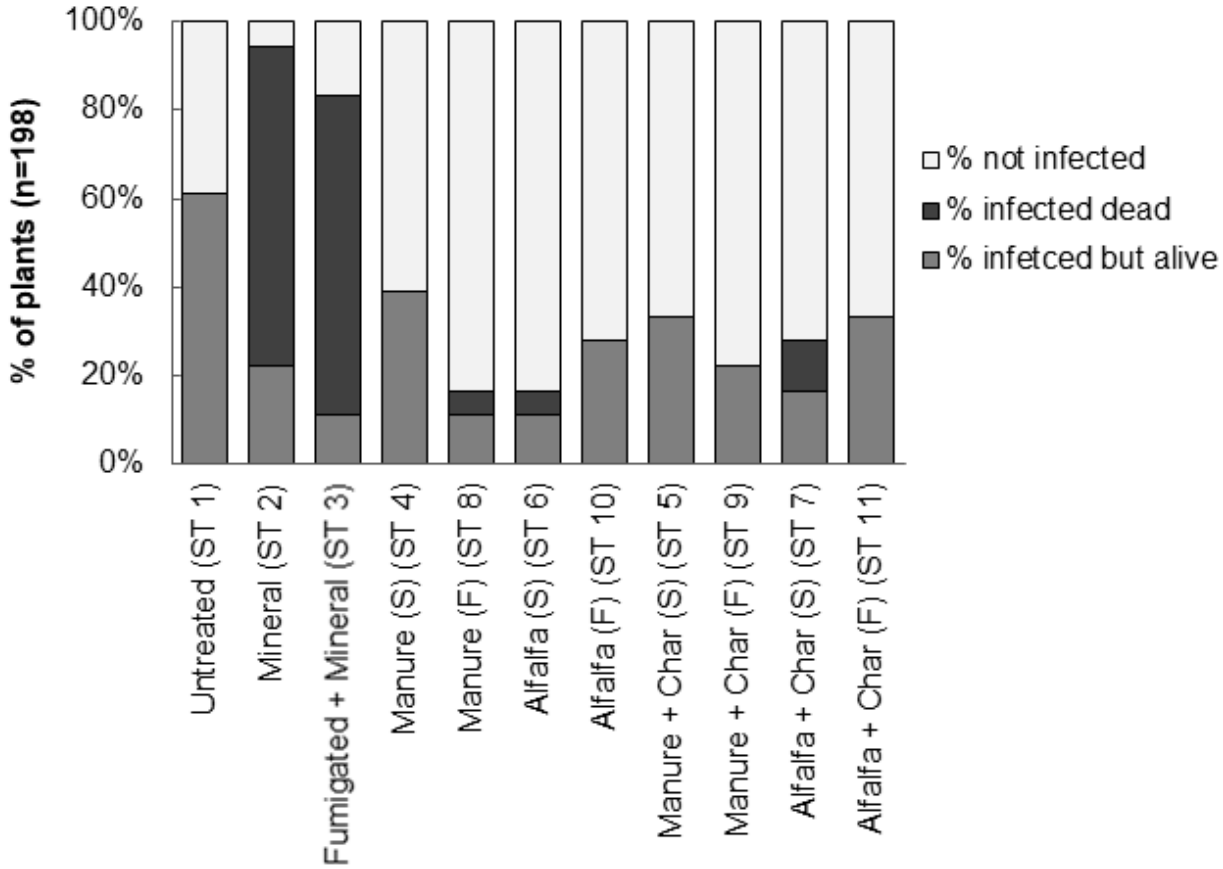

Table 1. Pearson correlations coefficients for chemical and microbiological parameters with damping off index (DOI) for *R. solani* – tomato and *S. sclerotiorum* – lettuce, percentage of mortality for *F. oxysporum* f. sp. *raphani* – lettuce, infected plants and percentage of mortality for TSWV – tomato.

	DOI (*R. solani* - tomato)	**DOI (*S. sclerotiorum* - lettuce)**	**Dead plants (*F. oxysporum* f. sp. *raphani* - lettuce)**	**Infected plants (TMWS - tomato)**	**Dead plants (TMWS - tomato)**
Chemical parameters					
pH	0.80 **	-0.15	-0.97**	-0.83**	-0.95**
EC (µS cm^{-1})	-0.78**	0.29	0.98**	0.85**	0.99**
Organic C (g kg^{-1})	0.10	-0.65*	-0.47	-0.52	-0.52
Total N (g kg^{-1})	-0.33	-0.72*	-0.05	-0.20	-0.06
C/N ratio	0.63*	-0.12	-0.73*	-0.64*	-0.81**
N-NO_3^- (mg l^{-1})	-0.81**	-0.32	0.62*	0.41	0.68*
N-NH_4^+ (mg l^{-1})	-0.51	0.47	0.66*	0.59	0.74**
Total limestone (g kg^{-1})	0.57	-0.41	-0.87**	-0.73*	-0.91**
Active limestone (g kg^{-1})	0.44	-0.15	-0.53	-0.62*	-0.47
CEC (meq $100g^{-1}$)	0.70*	-0.49	-0.93**	-0.84**	-0.96**
P_2O_5 (mg kg^{-1})	-0.27	-0.72*	0.02	-0.12	-0.07
K^+ (meq $100g^{-1}$)	0.06	-0.73*	-0.52	-0.63*	-0.44
Mg^{2+} (meq $100g^{-1}$)	-0.68*	0.40	0.80**	0.76**	0.77**
Ca^{2+} (meq $100g^{-1}$)	0.80**	-0.36	-0.95**	-0.81**	-0.98**
Na^+ (meq $100g^{-1}$)	-0.50	0.24	0.57	0.49	0.48
Microbiological parameters					
FDA (abs 490nm)	0.58	-0.60	-0.87**	-0.91**	-0.90**
Biolog EcoPlates™ (AWCD)	0.66*	-0.28	-0.72*	-0.80**	-0.64*

Significant differences at the 0.05 (*) and 0.01 (**) are reported.

Table 2: Pearson correlations coefficients for bacteria richness, diversity and relative abundance at genus level with damping off index (DOI) for *R. solani* – tomato and *S. sclerotiorum* – lettuce, dead plants for *F. oxysporum* f. sp. *raphani* – lettuce, infected plants and dead plants for TSWV – tomato. Only correlation with most abundant bacterial taxa are reported

	DOI (*R. solani* - tomato)	DOI (*S. sclerotiorum* - lettuce)	Dead plants (*F. oxysporum* f. sp. *raphani* - lettuce)	Infected plants (TMWS - tomato)	Dead plants (TMWS - tomato)
Synthetic parameters					
Chao1	0.69*	-0.19	-0.71*	-0.58	-0.74**
Shannon	0.66*	-0.49	-0.84**	-0.75**	-0.89**
Bacterial taxa					
Acidobacteria; Acidobacteria	0.68*	-0.16	-0.66*	-0.52	-0.76**
Acidobacteria; Chloracidobacteria	0.66*	0.08	-0.68*	-0.38	-0.75**
Acidobacteria; Solibacteres	0.67*	-0.22	-0.81**	-0.66*	-0.88**
Actinobacteria; Actinobacteria	0.16	-0.24	-0.02	-0.18	-0.01
Bacteroidetes; Flavobacteria	-0.58	0.48	0.75**	0.67*	0.81**
Bacteroidetes; Sphingobacteria	-0.77**	-0.14	0.64*	0.44	0.64*
Chloroflexi; Anaerolineae	0.71*	-0.12	-0.87**	-0.63*	-0.92**
Chloroflexi; Chloroflexi	0.09	-0.03	-0.37	-0.25	-0.27
Cyanobacteria; Chloroplast	-0.50	-0.13	0.44	0.22	0.38
Cyanobacteria; Nostocophycideae	0.40	0.23	-0.34	-0.10	-0.40
Cyanobacteria; Oscillatoriophycideae	0.64*	0.34	-0.29	-0.08	-0.34
Firmicutes; Bacilli	0.09	-0.35	0.02	-0.18	0.00
Gemmatimonadetes; Gemmatimonadetes	0.44	-0.18	-0.24	-0.19	-0.39
Nitrospirae; Nitrospira	0.59	-0.60	-0.72*	-0.76**	-0.79**
Planctomycetes; Planctomycea	-0.12	-0.38	0.09	-0.17	0.03
Proteobacteria; Alphaproteobacteria	-0.41	-0.48	0.19	0.03	0.27
Proteobacteria; Betaproteobacteria	0.15	-0.07	-0.21	-0.35	-0.12
Proteobacteria; Deltaproteobacteria	0.68*	-0.35	-0.82**	-0.77**	-0.87**
Proteobacteria; Gammaproteobacteria	-0.72*	0.15	0.93**	0.73*	0.93**
Verrucomicrobia; Verrucomicrobiae	0.09	-0.03	-0.50	-0.35	-0.44

Significant differences at the 0.05 (*) and 0.01 (**) are reported.

General conclusion

Soil sickness represents a condition in which the long-term use of non-sustainable agricultural practices causes changes in the physical, chemical and biological properties of soils that, in turn, negatively affects plant vegetative and reproductive performances. By an extensive analysis of literature, we found that soil sickness is pervasive in agro-ecosystems, occurring in 111 cultivated plants belonging to 41 taxonomic families. To explain the phenomenon of soil sickness, three main hypotheses have been proposed, including soil nutrient depletion or imbalance, build-up of soilborne pathogens coupled with shift in the composition of soil microbial community composition, and presence of phytotoxic and autotoxic compounds. Starting from a detailed analysis of mechanisms it was previously suggested that all proposed hypotheses have as common origin, i.e. the alteration of organic matter cycle caused by intensive agricultural practices.

Based on this consideration, in the present thesis different organic management strategies, in terms of organic matter type and application frequency, were used in order to recover a soil affected by soil sickness. Soil was subjected for two years to 11 different treatments including two ordinary soil managements, eight organic amendment treatments and one untreated soil as the control. At the end of each year, cumulated crop production of *Eruca sativa*, soil properties and soil microbiota were evaluated. Compared to the use of ordinary managements, the beneficial effects on soil properties and microbial community derived by the use of organic amendments were evident already after one cyear of conditioning. In detail, pH values near the neutrality, high soil organic carbon content and good level of soil aggregation, as well as an improvement in soil microbial functionality, richness and diversity were observed in soil treated with organic amendments, especially when easily decomposable materials rich in labile carbon and organic nitrogen (i.e., alfalfa plus glucose) were applied at high rate once a year. In contrast, cumulated crop production at the end of the first year was higher in soil with ordinary managements than in soil with application of organic materials. However, during the second year of soil conditioning, an increase in productivity and quality of the crop was observed in soil treated with organic materials as compared to the soil subjected to conventional management. Finally, soil conditioned for two years was used to evaluate the effects that ordinary and organic management strategies had in the disease suppression of soilborne phytopathogenic fungi and viruses. Application of organic amendments, by positively affecting soil properties and soil microbiota, showed a restoration of natural soil suppressivines against soilborne pathogens (i.e., *Sclerotinia sclerotiorum* and *Fusarium oxysporum* f. sp. *raphani*). Surprisingly, this study reports for the first time that the use of organic matter reduces the incidence of Tomato spotted

wilt virus infection, as well as the mortality of infected plants, probably by the induction of systematic resistance.

In conclusion, this study revealed that applications of organic materials have an immediate positive effect on soil fertility as well as on soil microbiota, while the increase of crop productivity are of longer-term nature. In addition, the positive effect that organic amendments have on microbial communities, including their abundance, diversity and richness of the several taxa, results in a recovery of the natural soil suppression against soilborne pathogens and the induction of plant resistance against airborne pathogens like viruses. However, the effects on crop production, soil fertility and disease suppression varied depending on quality, amount and frequency of application of organic matter. Therefore, future studies that include different combinations of organic amendment types and application frequencies, as well as different soil types, crop species and pathosystems, are needed to better understand the role of organic matter as a means to recover of soils affected by soil sickness.