Learning from nature: using plant-soil feedback principles to improve growth and health of a horticultural crop

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Chapter 1

General introduction

The influence of plants on soil

Plants are the main primary producers in terrestrial ecosystems. As providers of resources, they can significantly influence soil biota. Through releasing low-molecular-mass compounds (*i.e.* sugars, amino acids and organic acids), polymerized sugars (*i.e.* mucilage), root border cells and dead root cap cells, a plant creates a unique environment in its rhizosphere (Philippot et al. 2013; Badri et al. 2009). These compounds can attract or inhibit the growth of symbionts and pathogens in the rhizosphere (Bais et al. 2006). Plant identity is an important determinant of the composition and concentration of these root exudates, and thus an important determinant of the rhizosphere microbiome, which is defined as the microorganisms live in the rhizosphere of a particular plant (Bais et al. 2006; Bardgett and Wardle 2010; Bulgarelli et al. 2013). For example, *Acidobacteria* is one of the most abundance phyla in the rhizosphere of the plant *Mannillaria carnea*, while *Acidobacteria* are rarely detected in the rhizosphere of *Deschampsia antarctica* and *Colobanthus quitensis* (Torres-Cortés et al. 2012; Teixeira et al. 2010). Moreover, plants vary in net primary productivity (NPP), and hence in the amount of resources that enter the soil. Plant litter quantity, for example, can play an important role in structuring the soil microbial community, and can influence the rate of processes that occur in the soil, such as soil C sequestration and soil enzyme activity (Binkley and Giardina 1998; Kuzyakov and Blagodatskaya 2015; Tian and Shi 2014). These effects of plant litter on the bulk soil would also influence the composition of the plant rhizosphere microbiome (Pérez-Jaramillo et al. 2016). The influence of a plant on its rhizosphere microbiome is a dynamic process. When under attack by enemies, plants can also modify their rhizosphere microbiomes to increase the defense against the attack. For example, *Arabidopsis thaliana* recruits beneficial groups of rhizobacteria when under attack by a foliar pathogen (Rudrappa et al. 2008). Several studies reported that when under attack by soil pathogens, plants can also recruit beneficial microbes, which can act as antagonists to these soil pathogens (Bakker et al. 2018; Berendsen et al. 2018; Mavrodi et al. 2012). The influence of a plant on its rhizosphere microbiome may also depend on the genotype and the age of the plant (Chaparro et al. 2014; Wagner et al. 2016). All this makes the plant that grows in the soil an important determinant of the composition of its rhizosphere soil community (Bardgett and Wardle 2010).

The influence of soil on plants

Soil biota, in turn, also influence the performance of plants. Specific groups of soil microbes such as plant growth promoting bacteria, or arbuscular mycorrhizal fungi, promote plant growth and protect plants from pathogen attacks. Nitrogen-fixing rhizobia (such as *Rhizobium* and *Bradyrhizobium*) assist plants in uptaking nitrogen, and mycorrhizal fungi can facilitate phosphorus uptake of plants (Peix et al. 2015; Richardson et al. 2009). Mycorrhizal fungi can also translocate other nutrients and minerals from soil to plants (Gianinazzi et al. 2010; Johnson and Graham 2013). The mechanisms by which soil

microbes protect plants under biotic stress such as pathogen attack, include releasing antibiotics that can suppress soil pathogens (Haas and Défago 2005; Lugtenberg and Kamilova 2009), competition with soil pathogens for nutrients and microsites (Raaijmakers et al. 2009), hyperparasitism on soil pathogens (Druzhinina et al. 2011) and inducing systematic resistance in plants (van Loon et al. 2007). *Pseudomonas* and *Bacillus* are examples of beneficial bacteria, *Trichoderma*, *Gliocladium*, *Piriformospora* are fungi with beneficial functions for plants (Mendes et al. 2013).

Soil-borne pathogens are another major group of microbes that significantly influence plants. Root feeding nematodes and fungi (both the true fungi and fungi like oomycetes) are important soil-borne pathogens for plants (Mendes et al. 2013; Raaijmakers et al. 2009). In the soil, fungi and oomycete pathogens often persist in a dormant stage and become active after they encounter their favorable abiotic environment or cues from their host plants. Phenolic compounds from root exudates, such as gallic acid, coumaric acid and cinnamic acid, can stimulate the germination of soil pathogens in low concentrations (Wu et al. 2008; Zhang et al. 2012). Well-known fungal pathogens are *Fusarium oxysporum*, *Verticillium dahlia*. Oomycete fungi produce motile zoospores that can swim to the plant root for infection. Oomycete pathogenic fungi such as *Phytophthora infestans*, *Hyaloperonospora arabidopsidis*, and *Pythium ultimum* are among the most widely studied (Kamoun et al. 2015). Plant parasitic nematodes either feed on the root exterior, penetrate and move into root interior, or develop a feeding site inside the root and reproduce (Mendes et al. 2013). These nematodes are free living in the soil, and their sensory organs enable them to move to nutrient sites or host plants based on chemical cues (Mendes et al. 2013).

Plant-soil feedback

'Plant-soil feedback' refers to the changes in soil communities caused by a plant that in turn influence another plant that grows later in this soil (Bever et al. 1997; van der Putten et al. 2013). Plant-induced changes in soil communities can affect the growth of individual plants, but also the temporal dynamics of vegetation and hence the succession of plant species or the structuring of plant communities (Bardgett and Wardle 2010). Typical plant-soil feedback experiments have two phases, the conditioning phase, in which single or multiple plant species are used to condition the soil, and the feedback phase, in which target plant species or plant communities grow in the conditioned soil (van der Putten et al. 2013). There are mainly two ways to measure the feedback effects, one is to compare plant performance in sterilized vs non-sterilized soil (*i.e.* the effect of the whole community), the other is to compare plant performance in own vs other soil (*i.e.* the effect of species-specialized soil biota) (Brinkman et al. 2010). If the performance of the succeeding plant species is promoted by the previous plant species via influencing the soil, this is termed positive plant-soil feedback and the reverse is termed negative plant-soil feedback

(van der Putten et al. 2013). Moreover, if the succeeding plant species is the same as the preceding plant species, the feedback loop is termed conspecific plant-soil feedback, and if the succeeding plant species is not the same as the preceding plant species, it is termed heterospecific plant-soil feedback (van der Putten et al. 2013). It is well-known that plant-soil feedback effects are plant species-specific (van de Voorde et al. 2011), and most plant species suffer from negative conspecific feedbacks (Kulmatiski et al. 2008; Petermann et al. 2008). This indicates that a plant will grow better in soil conditioned by other plant species than in soil in which the same species has been grown (Cortois et al. 2016; van de Voorde et al. 2011).

Plant-soil feedbacks in agriculture

For more than 1000 years, humans have been aware of the importance of plant-soil feedbacks in agriculture. In particular, negative conspecific plant-soil feedback effects have received considerable attention from farmers. For example, long-term mono-cropping leads to the loss of crop yield because of the build-up of pathogens and nutrient depletion in the soil. Fruit trees were subjected to replanting failure when they were planted in soil where conspecific trees had been grown. Crop rotation systems have been developed to increase the establishment and productivity of crops in the soil (van der Putten et al. 2013; Dias et al. 2015). However, until now, a limited number of crops has been used in crop rotations. One group of plants that is widely used in rotations is leguminous plants, which not only increase the abundance of nitrogen-fixing rhizobia, but also increase the abundance of arbuscular mycorrhizal fungi in the soil (Vukicevich et al. 2016). This is because high phosphorus costs during fixation of nitrogen, make legumes depend on arbuscular mycorrhizal fungi at the nodulation sites (Scheublin et al. 2004). However, legumes can also have negative effects on disease-suppressive bacteria (Latz et al. 2012, 2015), which could be because root defense compounds, such as saponins, are higher in the legumes than in other plant species (Osbourn 2003). Brassica species are also widely used because of their beneficial effects on soil microbial communities. For example, *Brassica napus*, and *Brassica juncea* are known to increase the abundance of disease-suppressive bacteria and beneficial fungi such as *Trichoderma* in the soil (Mazzola et al. 2015; Berg et al. 2002; Hollister et al. 2013; Galletti et al. 2008). Typically, only cash crops or soil-conserving cover crops are included in the rotation system, and so far, these studies have been conducted only in agricultural soils. Recourses from natural ecosystems are often neglected in agricultural practices, indicating the huge gap between ecological knowledge and current farming practices (Weiner 2017; Dias et al. 2015).

Learning from nature

Recent papers (*e.g.* Mariotte et al. 2017; Pineda et al. 2017; Vukicevich et al. 2016) all highlight the importance of incorporating the soil and plant species from natural ecosystems for a more sustainable agriculture. Management practices in agricultural systems such as herbicide use, fungicide use, tillage and fertilization often lead to low biodiversity and simplified biotic interactions in the soil, while soils from natural ecosystems are typically much more diverse and with complex biotic interactions occurring within the soil communities (Mariotte et al. 2017). For example, in agricultural soils, tillage and fertilization typically decrease fungal biomass and disrupt arbuscular mycorrhizal fungal networks, resulting in the nitrogen leaching from soil (de Vries and Bardgett 2012). In contrast, soils from natural ecosystems often have higher abundance of and more diverse arbuscular mycorrhizal fungal communities (Holland et al. 2016). Moreover, compared with agricultural soils, soils from natural areas often poses greater suppression against soil pathogens (Garbeva et al. 2006), for example, the abundance of entomopathogenic fungi is often higher in these soils (Meyling et al. 2009). Moreover, a soil community with complex interactions is often more stable than the one with simple interactions when under abiotic or biotic pressures (Orwin and Wardle 2005; Griffiths and Philippot 2013) and a soil with high biotic diversity often reduces the possibility of the infection by soil pathogens to plants (van Elsas et al. 2012).

In contrast to many domesticated crops, many wild plant species have "host control" over their soil microbiome, *i.e.* they have traits that have evolved to recruit beneficial microbes for symbiosis, reward beneficial microbe genotypes, and exclude or sanction ineffective symbiosis (Kiers et al. 2003, 2011; Bakker et al. 2014; Sachs et al. 2010). Domesticated crops are selected for high yields, but in this selection process they may have lost the capability of host control and to shape their microbiome. Hence, they are sensitive to pathogen infection from soils (Mueller and Sachs 2015). Because of the traits and co-evolution mechanisms of wild plant species with the microbial communities, their microbiome may contain highly diverse and beneficial microbes that can be used to improve the productivity and sustainability of domesticated crops. This has been demonstrated by studies that found the root-associated microbes from wild relatives of crops can increase the ability of crops to cope with biotic (Santhanam et al. 2015) and abiotic stresses (Zachow et al. 2014). However, exploring the potential of wild plant species for agricultural benefits should move beyond only the wild relatives of domesticated crops, because other wild plant species also show beneficial interactions with soil microbes that can be potentially used to improve the yields of crops. Some wild plant species such as the grass *Lolium perenne*, for example, can enrich the abundance of bacteria that produce biocontrol compounds (Latz et al. 2015). The grass *Andropogon gerardi* enriches the abundance of mycorrhizal fungi in the soil (Hetrick et al. 1988), while another wild grass species, *Holcus lanatus* allocates more carbon to soil bacteria and *Actinomycetes* compared with forb and legume species (Ladygina and Hedlund 2010). Finally, the grass species *Anthoxanthum odoratum* stimulates the abundance of mycorrhizal fungi in the soil (De Deyn et

al. 2011). Such effects of wild plant species on soil microbiomes could provide potential benefits, such as disease resistance to soil pathogens, to crops that grow later in this soil. An important challenge is now to make use of these beneficial co-evolved plant-soil interactions of wild plant species to improve the productivity and sustainability of commercial crops.

Soil inoculation

Soil inoculation studies demonstrated that the effects of soil microbiomes are transferable by inoculating a small amount of donor soil to a recipient soil. For example, inoculating small amounts of soil collected from natural plant communities steered the recipient (agricultural) soil community to a more natural state, and subsequently, led to the establishment of target plant species, which resembled those found in natural communities (Wubs et al. 2016; Carbajo et al. 2011). Inoculating soil microbiomes related with later or early flowering time to the new plant host altered the flowering time of the plant into later and early directions, respectively (Panke-Buisse et al. 2015). Moreover, soil with disease-suppressive properties can be successfully transplanted and remains effective in the recipient soil even when only 5-10% is transplanted (Raaijmakers and Mazzola 2016; Mendes et al. 2011). The transferrable effects of soil microbiomes from the donor soil to the recipient soil could be that soil inoculation enhances the efficiency of the establishment of microbes in the recipient soil compared with inoculation of a single or a few beneficial microbial strains. Inoculating single or multiple microbial stains have often been reported to be insufficient as the microbes do not survive or fail to compete with native soil microbes (Gómez Expósito et al. 2017; Alabouvette et al. 2009; Gadhave et al. 2016). A recent study has pointed out that in order to maximize the plant growth promoting effects of beneficial microbial strains on plants, the microbes that play pivotal roles in organizing and keeping the composition of the beneficial microbiome are also important (Toju et al. 2018). This study further highlights the advantage of inoculating entire soil microbiomes as it can maximize the beneficial functions of certain microbial species by keeping the complex interactions between all the soil biota (Schlatter et al. 2017). Thus, if a plant species is known to create a positive soil feedback effect on a focal crop, disease suppressive soil could be created by growing this plant species first in soil, and then transferring this soil to the soil in which the crop is grown by soil inoculation.

Interactions between plant-conditioned soil communities

It is well-known that plant-soil feedback effects are plant species-specific, and an important question is whether there are synergistic, additive or antagonistic interactions between different plant-conditioned soil communities when mixed. Plant-soil feedback studies, which have tested the effects of mixtures of conditioned soils, are often carried out within in a "spatial heterogeneity" context and with naturally co-

occurring plants. These studies have been conducted to examine how spatial heterogeneity in soil resources and soil biota that is common in natural environments (because different plant species grow together but in each one conditions its own local soil) influences plant growth (Xue et al. 2018; Hendriks et al. 2015; Burns et al. 2017; Wubs and Bezemer 2016). In these studies, spatially heterogeneous soils were compared with spatially homogeneous soils and the results indicate that the net effects of mixing soils may not be additive (Xue et al. 2018; Hendriks et al. 2015; Burns et al. 2017; Wubs and Bezemer 2016). If the positive or negative effects of several conditioned soil inocula on the growth of a crop are known, will the effect of one conditioned soil inoculum be reduced by mixing it with an inoculum that has a different effect, and what will happen when two inocula with positive effects are mixed? Mixing soil microbial strains, such as beneficial or pathogenic strains, have been reported to interact both synergistically (Khan and Siddiqui 2017; Alizadeh et al. 2013) and antagonistically (Schisler et al. 1997; Johnson and Littrell 1970) on the inoculated plants. However, mixing two soil communities will be much more complex than mixing microbial strains, as the net effect of the mixture on plants may depend on both the interactions between different groups of soil microbes and also how they interact with the focal crop. Recently two studies, which tested the effects of homogenously mixing soil communities, found that two soil communities interacted synergistically in terms of soil pyrene removal efficiency (Wang et al. 2018), and agricultural land restoration (Wubs et al. 2018). Based on the results from these studies, we may expect that when mixing two soils, the effect of their mixture will be better than the sum of the effects of the two soils when inoculated separately. If this effect is also observed in agricultural systems, this means that the positive effect of inoculation with a beneficial soil may be strengthened by mixing it with another positive one.

Temporal dynamics of soil inoculation effects

Plants can leave legacies in the soil that have long-term influences on the soil microbial communities, thus can continue to influence other plants grow later in the soil (Wurst and Ohgushi 2015). This phenomenon has been observed both in natural (Kulmatiski et al. 2006) and agricultural systems (Detheridge et al. 2016). However, to achieve such long-term legacy effects may also require a long-term growth period of the preceding plant species in the soil (Kulmatiski and Beard 2011). In a horticultural system, in which crops are harvested at the end of every growth cycle, will the inoculated soil community in the beginning of the cultivation still influence the crop in the second or later growth cycles? Wubs and Bezemer (2018) reported that the conditioning plant species that grew in the first phase still influenced the plant species in third phase even though there was one other plant species growing in the soil in an intermediate phase. Thus, we may expect that if a beneficial microbiome has been inoculated into the soil at the beginning of the first growth cycle, the inoculated soil community may continue to influence the crop during later growth cycles. However, monocropping may decrease

the abundance of beneficial microbes and increase the abundance of pathogenic microbes in the soil, which subsequently leads to a more negative effect on the succeeding crop with increasing growth cycles (Sanguin et al. 2009; Zhou et al. 2016; Packer and Clay 2004). Therefore, although it is likely that the inoculated beneficial microbiome will continue to influence the crop in the second growth cycle, it is unknown whether the effect of the inoculated beneficial microbiome will remain positive or not. The net effect of the inoculated microbiome on the crop may depend on how fast the negative conspecific feedback effects of the crop build-up in the soil, and also depend on the interactions between the beneficial microbiome and the conspecific feedback effect of the focal crop.

Root-associated microbiome

Plant-soil feedback often uses a black box approach where the net effect of a plant, via the soil on another plant is recorded but without knowing what changed in the soil (van der Putten et al. 2013). However, apart from knowledge about the effects of inoculating soil conditioned by another plant species on crop performance, it is also important to identity the microbiome that has successfully established in the soil and that interacts with the crop. The root-associated microbiome includes two compartments: the rhizosphere (microbes surrounding the roots) and the endosphere (microbes within the roots) (Lundberg et al. 2012; Fitzpatrick et al. 2018). Compared to the microbiome in the bulk soil, the root-associated microbiome has more access to resources (root exudates) and typically contains a much larger population of microorganisms (Foster et al. 1983; Bakker et al. 2013). The composition of microbiota that are associated with plant roots can be greatly different from the composition of microbiota in the bulk soil (Lundberg et al. 2012; Bakker et al. 2013). The activities of these microorganisms are essential for plant functioning as they assist in plant nutrient uptake and protection against pathogen attack (Bakker et al. 2013). Identifying the root microbiome of a plant can provide knowledge about the potential beneficial and pathogenic microbes that may play a role in the productivity of that plant species. For example, *Enterobacter* strains isolated from the roots of Poplar trees showed significant growth promoting effects when inoculating these *Enterobacter* strains to Poplar saplings (Taghavi et al. 2009). A *Gluconacetobacter diazotrophicus* strain isolated from sugarcane roots is identified as a beneficial strain to sugarcane through fixing nitrogen and synthesizing auxin for this plant species (Bertalan et al. 2009). The bulk soil can also be an important determinant of the root-associated microbiome of plants. The microbiome in the bulk soil serves as the microbial seed bank for the root microbiome of the plant (Philippot et al. 2013). Not only does the type of bulk soil influence the assembly and composition of the root microbiome of plants (de Ridder-Duine et al. 2005; Mendes et al. 2014), but also changes brought about in the bulk soil can significantly influence the composition of the plant root microbiome (Estendorfer et al. 2017; Hartman et al. 2018; Liu et al. 2018). For example, the addition of bio-fertilizer alters the microbial composition in the bulk soil, and this subsequently steers the root microbiome of the

plant into a disease suppressive state (Liu et al. 2018). Another important factor in influencing the composition of plant root microbiome is the order of the arrival of the microbial species (Toju et al. 2018). For example, some biocontrol microbes are only effective in suppressing soil pathogens when they colonize the host plant before the pathogens (Braun-Kiewnick et al. 2000; Werner and Kiers 2015). These early-colonized beneficial microbes will fully use the habitat and resources of plant roots and produce antibiotics to the pathogen, thus, creating barriers for the colonization of pathogens (Wei et al. 2015). Therefore, by inoculating plants with a beneficial microbiome, the chances of pathogen infection to the host plant will be reduced. Moreover, we may also expect that by inoculating plants with different starter microbiomes, the root-associated microbiome of the plant will be modified in different ways. To advance our understanding and provide information about the development of methods to use soils to manipulate crop growth and health, it is important to identify the microbial groups that are associated with crop growth and health, and how they respond to different soil treatments.

Chrysanthemum as a model system

Chrysanthemum is one of the major cut flower crops that is cultivated in soil in commercial greenhouses in the Netherlands, and also an important export product with an export value of more than €250 million in 2017. Dutch growers produce most chrysanthemum in all year-round greenhouses. These greenhouses are divided into different cropping compartments. Cuttings are imported into the Netherlands and rooted in peat blocks by nurseries companies. These rooted cuttings in the peat blocks are then planted in chrysanthemum greenhouses. The growth period of chrysanthemum from planting untill harvest (including flowering) lasts on average 11 to 12 weeks depending on the season, after the plants have been harvested, new chrysanthemum cuttings are planted in the soil again (van der Hoeven 1986). This mono-cropping of chrysanthemum in the greenhouse leads to the rapid build-up of pathogens in the soil, and to control soil-borne disease, the soil is disinfected by steaming (Thuerig et al. 2009; Tamm et al. 2010). During the steaming process, the soil is covered with plastic foil and heating to 70°C using water boilers. This soil steaming is generally executed with an interval of five growth cycles. This practice leaves the soil as an empty niche, in which pathogens will re-establish rapidly as their antagonists are absent. However, this regularly disinfected soil also makes it a perfect system for testing plant-soil feedback effects. Many plant-soil feedback studies take the approach of inoculating a small amount of conditioned soil into sterilized bulk soil. With this approach, the abiotic soil conditions are kept constant in all treatments, enabling to focus on plant-soil feedback effects mediated by soil biota (Brinkman et al. 2010).

Belowground diseases of chrysanthemum

Soil-borne diseases are difficult to control because they can survive in soil for long time in the absence of the host crop, they often have a wide range of hosts, chemical control is often not environmental

friendly (Dignam et al. 2016). Chrysanthemum suffers from soil-borne diseases caused by a wide range of pathogens. In this thesis, I test the effects of one oomycete pathogen and one plant parasitic nematode on chrysanthemum.

Pythium ultimum is a pathogenic oomycete, which infests both germinating seeds and roots of mature plants with a wide range of host plant species. *Pythium* spp are found in undisturbed soils (Hendrix and Campbell 1973) as well as in previously cultivated soils (Pettitt et al. 2011). In chrysanthemum, the symptoms of *Pythium* infection are black lesions that occur on stems near the soil line, stunted growth, reduced development of root systems and wilting (Reddy 2016). Although *Pythium* can quickly colonize organic matter present in the soil, this pathogen is a poor competitor and suffers in competition with other soil microorganisms (Awasthi 2015). A study that tested the soil suppression against *Pythium* found that many microbial parameters are negatively associated with *Pythium* growth rates, and that high microbial biomass and activity can induce soil suppression against *Pythium* growth in horticultural soils (van Os and van Ginkel 2001). Specific groups of fungi, such as *Trichoderma* and *Gliocladium* spp, and bacteria, such as fluorescent pseudomonads, *Burkholderia cepacia*, *Enterobacter cloacae* are effective antagonists against *Pythium* (Martin and Loper 1999). The horticultural practice of steaming soil will eliminate these antagonists and may facilitate colonization of *Pythium* on chrysanthemum (Knudsen et al. 2002).

Meloidogyne incognita is a sedentary root endoparasitic nematode, which causes root-knot disease of both cultivated and wild plant species. The infective stage of the nematode is the second stage juvenile (J2). The infective J2 penetrates the root and goes through three stages before it becomes an adult. The infection of *Meloidogyne* in the plant causes abnormal root galls (Siddiqui et al. 2014). The activity of *Meloidogyne* in roots kills root cells, and the root eventually starts to rot. The nematode can dwell in dead root tissues, and this can act as an infection source in the next growth cycle or season (Reddy 2016). The infection severity of *Meloidogyne* in roots can be characterized using the gall index, which describes the gall number and size at a categorical level (Dias et al. 2016). Aboveground symptoms of *Meloidogyne* on chrysanthemum are yellowing of leaves and stunted growth. Bacteria, such as fluorescent pseudomonads, *Bacillus thurigiensis*, and fungi, such as *Trichoderma harzianum*, *Purpureocillium lilacinum* are antagonists to *Meloidogyne incognita* (Berg 2009; Li et al. 2007; Silva et al. 2018). Plant species that belong to the family *Tagetes* also have antagonistic effects to *Meloidogyne incognita* because of the nematicidal compounds that are released by their roots (Chitwood 2002; Hooks et al. 2010).

Aboveground pests of chrysanthemum

Chrysanthemum suffers from many aboveground pests such as aphids, leaf miners, thrips, leaf folders and spider mites (Reddy 2016). Thrips is one of the major pests in chrysanthemum (Maniania et al. 2013; Anyango et al. 1992; Leiss et al. 2009), and one of the major pests worldwide in many vegetables and ornamental crops (Manners et al. 2013; Leiss et al. 2013; Badenes-Pérez et al. 2018). Increased resistance of chrysanthemum to thrips is related to the increase of its resistance to other pests, such as leaf miner and spider mite (Kos et al. 2014). In this thesis, I test the effect of Western flower thrips (*Frankliniella occidentalis*), a generalist herbivore, on chrysanthemum. *Frankliniella occidentalis* are sucking cell-feeders that live and reproduce on the flowers and leaves. Besides the direct negative effects of western flower thrips on chrysanthemum, it can also cause indirect damage to chrysanthemum via transmission of viruses and it can alter the appearance of the flowers (Reddy 2016). The life cycle of thrips consists of five stages: egg, larval, prepupal, pupal and adult. The eggs of thrips can be inserted into soft plant tissues, such as flowers, leaves and stems. The prepupal and pupal stages often develop on the soil or growth medium, and the pupation develops on the plant. Thus, thirps are in contact with the soil during the pupal stage, and soil microbes may influence thrips survival or development (Manners et al. 2013). The adult thrips are weak flyers, usually taking short flights from leaf to leaf or plant to plant, but they can disperse rapidly in the greenhouse (Manners et al. 2013). Predatory mites (Gerson and Weintraub 2007; McMurtry et al. 2013; Manners et al. 2013) and entomopathogenic microbes (Lacey et al. 2015; Maniania et al. 2003) are two widely studied biological control agents of thrips in greenhouses.

Plant species used in the conditioning phase

In this thesis, I use 36 wild plant species that are native to the Netherlands and that occur in temperate grasslands, and one domesticated plant to condition the soil in the monoculture. Some of these selected conditioning plant species have been reported to either have antagonistic effects on soil-borne diseases or to promote beneficial microbes, and the domesticated crop has antagonistic effect on plant parasitic nematodes (Table 1.1). I grew these plants in soil collected from a natural grassland. In natural ecosystems, microbial communities are more diverse than in agricultural systems, and plants and microorganisms have co-evolved. These co-evolved microorganisms may have a more positive effect on plants (Philippot et al. 2013). Therefore, growing wild plant species in their native soil may lead to good functional microbiomes so that the inocula from these soils can be used to improve the performance of crops.

Table 1.1 Influence of conditioning plant species (36 wild plant species and one domesticated crop) on soil biotic and abiotic processes and their current uses in agriculture.

Species	Functional group	Soil effects	Agricultural use	Reference
Agrostis capillaris	Grass	increases soil nitrification rates; weak association with mycorrhizal fungi	cover crop	Veresoglou et al. 2011; Weigelt et al. 2003
Agrostis stolonifera	Grass			
Anthoxanthum odoratum	Grass	increases the abundance of mycorrhizal fungi; increases soil microbial biomass and respiration		De Deyn et al. 2011; Innes et al. 2004
Bromus hordeaceus	Grass	higher nitrogen flow to mycorrhizal fungi	cover crop	Costello 2010; Cheng and Baumgartner 2004
Festuca filiformis	Grass			
Festuca rubra	Grass	suppresses a plant parasitic nematode	cover crop	Vrain et al. 1996
Holcus lanatus	Grass	increases microbial biomass and respiration; allocates more carbon to bacteria; increases *Actinomycetes*; promotes a bacteria-dominated soil food web; reduces the number of bacteria-feeding nematodes		Innes et al. 2004; Ladygina and Hedlund 2010; Witt and Setälä 2010; Saj et al. 2009
Lolium perenne	Grass	increases the abundance of antibiotics-producing bacteria; increases soil nitrification rates;	cover crop	Latz et al. 2015; Weigelt et al. 2003; Brant et al. 2009; Mauro et al. 2015
Phleum pratense	Grass			
Achillea millefolium	Forb	suffers from soil fungi	cover crop	Dastgheib et al. 1999; Šmilauer and Šmilauerová 2000
Arabidopsis thaliana	Forb		model plant to indicate the breeding of crops	Huot et al. 2014; Arabidopsis Genome Initiative 2000
Arnica montana	Forb	strong dependence on mycorrhizal fungi (more mycorrhizal fungi?)		Jurkiewicz et al. 2010; Wardecki et al. 2015
Campanula rotundifolia	Forb	strong dependence on mycorrhizal fungi (more mycorrhizal fungi?)		Stevens et al. 2012
Capsella bursa-pastoris	Forb			
Carum carvi	Forb			
Centaurea jacea	Forb			
Crepis capillaris	Forb			
Galium verum	Forb		cover crop	Miglécz et al. 2015
Hypericum perforatum	Forb			
Hypochaeris radicata	Forb			
Jacobaea vulgaris	Forb	chemical compounds extracted from roots have inhibitory effects on plant-associated fungi		Hol and van Veen 2002
Leucanthemum vulgare	Forb	increases the abundance of mycorrhizal fungi		Reidinger et al. 2012; Bharadwaj et al. 2007
Matricaria recutita	Forb			
Plantago lanceolata	Forb	increases the abundance of mycorrhizal fungi; inhibitory effect on soil N mineralization	cover crop	Šmilauer 2001; Dietz et al. 2013; Miglécz et al. 2015
Prunella vulgaris	Forb	increases the abundance of mycorrhizal fungi		Veresoglou et al. 2011
Rumex acetosella	Forb	low abundance of bacteria in its root		Vale et al. 2005;
Tagetes minuta	Forb; A domesticated crop	antagonisitic effect on plant parasitic nematodes	cover crop	Hooks et al. 2010; Kimpinski et al. 2000; Natarajan et al. 2006; Sturz and Kimpinski 2004
Tanacetum vulgare	Forb	antimicrobial effects of root extracts		Devrnja et al. 2017
Taraxacum officinale	Forb	reduces the diversity of non-mycorrhizal fungi; increases AMF abundance; improves soil aggregation		Becklin et al. 2012; Kabir and Koide 2000
Thymus pulegioides	forb			
Lotus corniculatus	Legume	fungi-dominated soil web; reduces the diversity of soil microbes; increases soil carbon and nitrogen stocks	cover crop	Witt and Setälä 2010; Li et al. 2013; De Deyn et al. 2009
Medicago sativa	Legume	increases the nitrogen uptake of later crop; increases the abundance of soil bacteria and fungi	cover crop	Amossé et al. 2014; Chang et al. 2016; Zhao et al. 2015
Trifolium arvense	Legume			
Trifolium pratense	Legume	increases the nitrogen uptake of later crop; increases the abundance of mycorrhizal fungi	cover crop	Amossé et al. 2014; Veiga et al. 2013
Trifolium repens	Legume	increases soil carbon and nitrogen stocks; increases the nitrogen uptake of later crop; increases the diversity of *Pseudomonas*; increases the diversity of soil bacterial and fungal communities	cover crop	De Deyn et al. 2009; Chang et al. 2017; Li and Wu 2018; Amossé et al. 2014
Vicia cracca	Legume		cover crop	Goar 1934
Vicia sativa	Legume	suppresses plant parasitic nematodes; increases soil nitrogen; increases soil carbon liability	cover crop	Bayer et al. 2009; Novara et al. 2013; Hagan et al. 1998

Aim of this thesis

In this thesis, I test plant-soil feedback effects in the context of improving chrysanthemum growth and disease susceptibility to belowground diseases and aboveground thrips.

First, I test the plant-soil feedback effects of a large range of wild plant species on chrysanthemum. In this study, 37 plant species from three functional groups (grass, legume, forb) were used to condition a soil collected from a natural grassland. The effects of inoculation of these different soils conditioned by each of the plant species individually on chrysanthemum in presence and absence of a soil-borne pathogen *Pythium ultimum* were evaluated (**Chapter 2**). I examine the positive and negative effects of these inocula in terms of chrysanthemum growth, chrysanthemum susceptibility to *Pythium* infection, and measure how inoculation altered the levels of plant defense compounds in chrysanthemum leaves as an indication for the potential defense ability of chrysanthemum against aboveground pests such as thrips. Then, I analyze the generality of the effects of these inocula by comparing the effects within and between the functional groups of the conditioning plant species, and by analyzing whether the effects of these inocula depend on the phylogenetic distances of the conditioning plant species to chrysanthemum.

Second, I test the additivity of plant-soil feedback effects on chrysanthemum. Based on the previous study, I selected eight of the 37 plant species with negative or positive effects on chrysanthemum. In this study, monospecific conditioned soil inocula were pairwise mixed, and the effects of these mixtures and the monospecific soil inocula on chrysanthemum growth and leaf yellowness were tested (**Chapter 3**). I compare the observed effects of each mixed inoculum with the predicted effects of this mixed inoculum based on the effects of the monospecific soil inocula to infer whether the interactions between two soil communities are synergistic, antagonistic or additive. Further, I examine whether the differences between the effects of two soil communities influence their interactions when mixed.

Third, I test the carry-over effects of plant-soil feedback effects on chrysanthemum. The same eight plant species were used to condition natural grassland soil and these conditioned soils were inoculated into sterilized soil at the beginning of the first growth cycle. Chrysanthemum was then grown in these soils for two consecutive growth cycles. During the first growth cycle, a subset of the plants was exposed to either the soil pathogen *Pythium*, or the plant parasitic nematode *Meloidogyne*. In the second growth cycle, these plants were then again exposed to *Pythium* or to a second soil inoculum that is collected from a commercial chrysanthemum greenhouse and that contained a high density of *Meloidogyne* nematodes (**Chapter 4**). I test whether the soil inocula added in the beginning of the first growth cycle continue to influence chrysanthemum performance during the second growth cycle. Then, I analyze whether the direction of the effects of the different soil inocula changes during the two successive growth

cycles. Finally, I examine the effects of soil inocula on chrysanthemum growth and health with and without the disease treatments in each growth cycle to infer how soil inocula influence the disease susceptibility of chrysanthemum during the two successive growth cycles.

Forth, I test the possibility of using wild plant species and grassland soil to steer the soil from a commercial chrysanthemum greenhouse towards a more beneficial state for chrysanthemum. I examine how inoculation influences the composition of root microbiomes of chrysanthemum, and how it affects the growth of chrysanthemum, and the susceptibility to thrips. The soils used for conditioning were collected from a natural grassland or from a commercial greenhouse, eight wild plant species and chrysanthemum were used to conditioned the soils individually. The conditioned soils were inoculated in background soil that consisted of sterilized or live commercial greenhouse soil (**Chapter 5**). I describe the composition of the chrysanthemum root microbiome in different soil treatments and correlate changes in the root microbiome with chrysanthemum growth and its susceptibility to thrips.

Finally, I conclude with a general discussion and synthesis of my findings (**Chapter 6**). I compare the results of my studies with other studies in the field. I will highlight a number of questions that deserve further investigation and identify issues related to the practical implementation of the results.

Chapter 2

Plant–soil feedback effects on growth, defense and susceptibility to a soil-borne disease in a cut flower crop: species and functional group effects

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Abstract

Plants can influence the soil they grow in, and via these changes in the soil they can positively or negatively influence other plants that grow later in this soil, a phenomenon called plant–soil feedback. A fascinating possibility is then to apply positive plant–soil feedback effects in sustainable agriculture to promote plant growth and resistance to pathogens. We grew the cut flower chrysanthemum (*Dendranthema X grandiflora*) in sterile soil inoculated with soil collected from a grassland that was subsequently conditioned by 37 plant species of three functional groups (grass, forb, legume), and compared it to growth in 100% sterile soil (control). We tested the performance of chrysanthemum by measuring plant growth, and defense (leaf chlorogenic acid concentration) and susceptibility to the oomycete pathogen *Pythium ultimum*. In presence of *Pythium*, belowground biomass of chrysanthemum declined but aboveground biomass was not affected compared to non-*Pythium* inoculated plants. We observed strong differences among species and among functional groups in their plant–soil feedback effects on chrysanthemum. Soil inocula that were conditioned by grasses produced higher chrysanthemum above- and belowground biomass and less leaf yellowness than inocula conditioned by legumes or forbs. Chrysanthemum had lower root/shoot ratios in response to *Pythium* in soil conditioned by forbs than by grasses. Leaf chlorogenic acid concentrations increased in presence of *Pythium* and correlated positively with chrysanthemum aboveground biomass. Although chlorogenic acid differed between soil inocula, it did not differ between functional groups. There was no relationship between the phylogenetic distance of the conditioning plant species to chrysanthemum and their plant–soil feedback effects on chrysanthemum. Our study provides novel evidence that plant–soil feedback effects can influence crop health, and shows that plant–soil feedbacks, plant disease susceptibility, and plant aboveground defense compounds are tightly linked. Moreover, we highlight the relevance of considering plant–soil feedbacks in sustainable horticulture, and the larger role of grasses compared to legumes or forbs in this.

Key words: Chlorogenic acid, Chrysanthemum, Disease susceptibility, Plant–soil feedback, *Pythium ultimum*, Plant functional group, Phylogenetic distance

Introduction

Plants are the main primary producers in terrestrial ecosystems and as provider of resources, such as litter and root exudates, plants are important determinants of soil biota (Bever et al. 1997; Bardgett and Wardle 2010). These effects of plants on the soil may differ greatly between plant species as plants vary in the quality and quantity of litter and in the chemical composition of root exudates (Wardle et al. 2003; Bais et al. 2006; Bardgett and Wardle 2010). Moreover, via their effects on the soil, plants can influence other plants that grow later in the same soil, a phenomenon termed 'plant–soil feedback' (van der Putten et al. 2013). Plant–soil feedback effects can be positive, if the succeeding plant grows better in conditioned soil compared to a control soil, and negative, if the growth is reduced (van der Putten et al. 2013). Heterospecific plant–soil feedback (where one species influences the growth of another species) has been recognized as an important mechanism in plant competition and coexistence (Kulmatiski et al. 2008; van der Putten et al. 2013), and there is an increasing interest among ecologists to unravel the mechanisms and determine the generality of plant–soil feedback effects (van der Putten et al. 2013). Although negative conspecific feedbacks are the basis for crop rotation in agriculture, how heterospecific plant–soil feedback influences cultivated plant species is relatively poorly understood as most studies, so far, have focused on interactions among wild plant species (van der Putten et al. 2013; Dias et al. 2015; Detheridge et al. 2016).

Heterospecific plant–soil feedback effects may differ between plant functional groups such as grasses, forbs or leguminous plants (Bezemer et al. 2006; Kos et al. 2015). Legumes, as nitrogen fixers may increase nutrient availability for other plants, and thus may cause positive plant–soil feedback effects (Tilman et al. 1997; Harrison and Bardgett 2010). Similarly, grasses which have highly branched roots may provide a more suitable habitat for root-associated microbes that have beneficial effects on other plants (Bessler et al. 2009; Pérès et al. 2013; Latz et al. 2015). Clearly, an increase in root surface area that is often found in grasses could also lead to an increase in the abundance of plant antagonists such as root pathogens, but root pathogens of grasses are specialized on monocots, and it is unlikely they will negatively influence plants from another functional group (Cortois et al. 2016). Instead, roots of forb species that typically have higher phosphate contents than grass species are more susceptible to soil-borne pathogens (Laliberté et al. 2015; Zhang et al. 2016). Hence, forbs often host more pathogens than grasses, and are thereby more likely to have a negative feedback effect on later growing plants (Rottstock et al. 2014). As closely related species are more likely to share the same natural enemies and resources (Webb et al. 2006; Gilbert and Webb 2007), it is legitimate to hypothesize that heterospecific plant–soil feedback effects among closely related species are more negative than among more distantly related species (Brandt et al. 2009; Burns and Strauss 2011; Anacker et al. 2014; Mehrabi and Tuck 2015; Münzbergová and Šurinová 2015).

By growing in the soil, a plant may cause an increase in the density of pathogens in the soil, but at the same time, it may also increase beneficial microbes such as bacteria and fungi that promote plant growth, suppress pathogens or induce resistance in plants against herbivore or pathogen attack (Haas and Défago 2005; Pineda et al. 2010). Hence, plant–soil feedback effects could influence the susceptibility of a plant to soil pathogens or the disease or pest severity experienced by that plant. We are not aware of any work reporting how plant–soil feedback influences the susceptibility of a plant to soil pathogens, but several studies reported that conditioning of soil by a plant can influence the levels of aboveground herbivory experienced by another plant that grows later in that soil via the feedback effects on the composition and concentration of aboveground secondary compounds of the responding plant (Kostenko et al. 2012; Bezemer et al. 2013; Kos et al. 2015). Soil biota, such as root herbivores, nematodes, and (non-) pathogenic soil microbes can affect plant aboveground primary and secondary compounds (Bezemer et al. 2005; Soler et al. 2012; van de Mortel et al. 2012; Badri et al. 2013), and hence we may expect that plant–soil feedback effects on the susceptibility of a plant to soil diseases will also influence the concentration of aboveground defense compounds in that plant.

In the present study, we examine how plant–soil feedback effects of a wide range of plant species influence the growth and secondary chemistry of the commercial cut flower chrysanthemum and its susceptibility to the soil pathogen *Pythium ultimum*. *Pythium* causes damping off disease to a wide range of plants including chrysanthemum (Weller et al. 2002; Meghvansi and Varma 2015). Several studies have shown that high abundance and diversity of soil microbes can suppress *P. ultimum* (van Os and van Ginkel 2001; Yu et al. 2015). We examined in a greenhouse experiment the plant–soil feedback effects of 37 plant species belonging to three plant functional groups on chrysanthemum growth and disease susceptibility. We tested three hypotheses: (i) plant–soil feedbacks will not only influence plant growth, but also influence plant disease susceptibility and plant defense, (ii) soil conditioned by grasses and legumes will positively affect chrysanthemum growth and reduce disease severity relative to soil conditioning by forbs, (iii) species closely related to chrysanthemum will have a more negative effect on chrysanthemum growth than more distantly related species.

Materials and methods

Plant and pathogen material

The focal plant in our study is *Dendranthema X grandiflora* (Ramat.) Kitam. cv. Grand Pink [Chrysanthemum, syn. *Chrysanthemum X morifolium* (Ramat.) Hemsl., Asteraceae]. Chrysanthemum cuttings were provided by the breeding company FIDES by Dümmen Orange (De Lier, Netherlands). Chrysanthemum is one of the major cut flower crops that is cultivated in soil in greenhouses. In

commercial chrysanthemum greenhouses, the soil is disinfected regularly with hot steam to circumvent soil diseases. However, this practice also eliminates the (beneficial) microbial community in the soil and pathogens rapidly recolonize the soil after steaming (Thuerig et al. 2009; Tamm et al. 2010).

The soil–borne oomycete pathogen *Pythium ultimum* (Pythiaceae) was obtained from Wageningen UR Greenhouse Horticulture (Wageningen UR, Greenhouse Horticulture, Bleiswijk, Netherlands). *Pythium ultimum* was isolated from diseased chrysanthemum plants, and cultured on liquid V8 medium (200 ml of organic tomato suspension without added salt, 2 g CaCO3, and 800 ml water) at room temperature for 2 weeks. Then, the *P. ultimum* culture was blended in a mixer and filtered to obtain a solution with only oospores based on a modified protocol of van der Gaag and Wever (2005). The oospores concentration was determined by counting (Fuchs-Rosenthal chamber) the oospore number in 1 ml liquid suspensions under the microscope.

Experimental set-up

The experiment consisted of two phases. In the first phase, the conditioning phase, we used 37 plant species to condition soil by growing them in monocultures. In the second phase, the test phase, we measured the effects of the species-specific conditioned soils as inocula on the performance of chrysanthemum plants with and without *P. ultimum* addition.

Phase I: Conditioning phase

For the conditioning phase, 300 Kg soil was collected (5–20 cm deep) in November 2014 from a semi-natural grassland that was previously used to grow maize and where agricultural activities ceased in 1995 (Mossel, Ede, Netherlands). The collected soil was homogenized and sieved (1 cm mesh size) to remove coarse fragments and all macro-arthropods. Pots (13 cm × 13 cm × 13 cm) were filled with a homogenized mixture of field soil and sterilized field soil in a 1:1 ratio (total 1.6 Kg soil per pot). Part of the soil was sterilized by gamma irradiation (>25 K Gray gamma irradiation, Isotron, Ede, Netherlands).

Thirty-seven plant species were selected to create conditioned soils (Table 2.1). The species were classified as grasses (9 species), forbs (21 species), or legumes (7 species) (Table 2.1). Most species were wild species that are typical of natural grasslands in Netherlands. *Tagetes minuta* is a domesticated species that was included because of its known disease suppressive properties (Hooks et al. 2010). Seeds of the wild species were obtained from a wild plant seed supplier (Cruydt-Hoeck, Assen, Netherlands)

and *Tagetes minuta* seeds were obtained from a garden plant seed supplier (Vreeken seeds, Dordrecht, Netherlands). Seeds were surface sterilized in 3% sodium hypochlorite solution for 1 min, rinsed and germinated on sterile glass beads in a climate chamber at 20°C (16 h/8 h, light/dark).

Five 1-week-old seedlings were transplanted in monocultures in each pot (13 cm × 13 cm × 13 cm), with five replicate pots for each species. A set of five pots filled with field soil (without plants) was also kept in the greenhouse, and served as the "no plant" control for the test phase. In total, the conditioning phase comprised of 190 pots (monocultures of 37 plant species × 5 replicates + no plant pots × 5 replicates). The replicate pots of each species in the conditioning phase were kept separately throughout the experiment. Seedlings that died during the first week of the experiment were replaced. A few seedlings died after transplantation. Therefore, 2 week later, the number of seedlings in each pot was reduced to four. All pots were placed randomly in a greenhouse with 70% RH, 16 h 21° (day) and 8 h 16° (night). Natural daylight was supplemented by 400 W metal halide lamps (225 μmol $s^{-1}m^{-2}$ photosynthetically active radiation, one lamp per 1.5 m^2). The pots were watered regularly. Ten weeks after transplanting, plants were clipped and the largest roots were removed from the soil as they may act as a source for re-growing plants. Finer roots were left in the soil as the rhizosphere may include a major part of the microbial rhizosphere community. The soil from each pot was homogenized and stored in a plastic bag at 4°C (1 bag for each pot) until used in the test phase. These soils are called "soil inocula" hereafter.

Phase II: Test phase

For the test phase, 1 L pots (11 cm × 11 cm × 12 cm; length × wide × height) were filled with a homogenized mixture of 10% soil inoculum (plant species-specific conditioned soil) and 90% sterile soil (see above). Two controls were included in the test phase: 100% sterile soil and 90% sterile soil mixed with 10% field soil that was kept without plants in the greenhouse during the conditioning phase ("no plant" inoculum). Two chrysanthemum cuttings (without roots) were planted in each pot as preliminary work showed that not all cuttings establish properly with this method. Prior to planting, the soil in each pot was well watered and 100 ml half-strength Hoagland nutrient solution was added. The pots were placed on trolleys, each trolley had 48 pots and was tightly covered with a thin transparent plastic foil for 10 days to create a closed environment with high humidity that favors rooting. After 10 days, one of the chrysanthemum cuttings was removed from each pot. Plants were fertilized following grower's practice: half-strength Hoagland nutrient solution for the first 2 weeks, and single strength Hoagland solution during the following 2 weeks. For the last 2 weeks, the strength was increased to 1.6 mS/cm EC (electrical conductivity). The density of pots on each trolley was reduced 2 weeks after the start of the second phase to 32 pots per trolley so that there was 10 cm space between each pot.

Table 2.1. List of plant species used in the conditioning phase, their abbreviation used in the manuscript, family and functional group are also presented.

Species	Abbreviation	Family	Functional group
Agrostis capillaris	AC	Poaceae	Grass
Agrostis stolonifera	AS	Poaceae	Grass
Anthoxanthum odoratum	AO	Poaceae	Grass
Bromus hordeaceus	BH	Poaceae	Grass
Festuca filiformis	FF	Poaceae	Grass
Festuca rubra	FR	Poaceae	Grass
Holcus lanatus	HL	Poaceae	Grass
Lolium perenne	LP	Poaceae	Grass
Phleum pratense	PP	Poaceae	Grass
Carum carvi	CAC	Apiaceae	Forb
Achillea millefolium	ACM	Asteraceae	Forb
Arnica montana	ARM	Asteraceae	Forb
Centaurea jacea	CJ	Asteraceae	Forb
Crepis capillaris	CRC	Asteraceae	Forb
Hypochaeris radicata	HR	Asteraceae	Forb
Jacobaea vulgaris	JV	Asteraceae	Forb
Leucanthemum vulgare	LV	Asteraceae	Forb
Matricaria recutita	MR	Asteraceae	Forb
Tagetes minuta	TM	Asteraceae	Forb
Tanacetum vulgare	TV	Asteraceae	Forb
Taraxacum officinale	TO	Asteraceae	Forb
Arabidopsis thaliana	AT	Brassicaceae	Forb
Capsella bursa-pastoris	CB	Brassicaceae	Forb
Campanula rotundifolia	CR	Campanulaceae	Forb
Hypericum perforatum	HP	Hypericaceae	Forb
Prunella vulgaris	PV	Lamiaceae	Forb
Thymus pulegioides	THP	Lamiaceae	Forb
Plantago lanceolata	PL	Plantaginaceae	Forb
Rumex acetosella	RA	Polygonaceae	Forb
Galium verum	GV	Rubiaceae	Forb
Lotus corniculatus	LC	Fabaceae	Legume
Medicago sativa	MS	Fabaceae	Legume
Trifolium arvense	TA	Fabaceae	Legume
Trifolium pratense	TRP	Fabaceae	Legume
Trifolium repens	TR	Fabaceae	Legume
Vicia cracca	VC	Fabaceae	Legume
Vicia sativa	VS	Fabaceae	Legume

Five days after the transparent plastic foil had been removed, 3 ml of the oospore suspension (ca. 355000 oospores of *P. ultimum*) was added onto the soil next to the stem of each plant allocated to the disease treatment. For plants in the control treatment (non-*Pythium* inoculated), 3 ml water was added. In both

treatments, there were two replicate pots for each soil from the conditioning phase. Hence, the feedback phase comprised of 780 pots [(37 plant specific soil inocula + no plant soil inoculum) × 2 disease treatments × 5 soil replicates × 2 replicate pots + 100% sterile soil × 2 disease treatments × 10 replicates]. All pots were randomly arranged in a greenhouse compartment and kept under the same conditions as described for the conditioning phase.

Plant performance and disease susceptibility

Six weeks after disease inoculation, all plants were harvested. For each plant, the total number of leaves and the number of yellow leaves was recorded and plant yellowness was calculated as the proportion of yellow leaves. The third fully expanded leaf from the top of each plant was then clipped and stored at -80°C for chlorogenic acid analysis (see below). Plants were then clipped at soil level and roots were rinsed from the soil. Shoot and root biomass were oven-dried (60°C for 3 days) and weighed and the root/shoot ratio was calculated. The main symptom of *Pythium* infection is the reduced root system caused by root rot (Agrios 2005), and thus plant root/shoot ratio is used as an indicator of plant susceptibility to *Pythium*.

Analysis of chlorogenic acid

Chlorogenic acid acts as an important resistance factor in chrysanthemum against plant attackers such as herbivorous insects (Leiss et al. 2009). Chemical analysis was performed using high performance liquid chromatography (HPLC) with UV diode array detection following the procedure outlined by Olszewska (2007). Leaves were freeze-dried and finely ground. Ten mg of ground leaf material was then used for chemical analysis. Each leaf sample was extracted twice. In the first extraction, 1 ml 70% MeOH was added to each sample, vortexed for 0.5 min, then ultrasonicated for 30 min at 20°C, centrifuged for 10 min at 10000 rpm, and labeled. The extraction was repeated so that each sample was extracted by 2 ml 70% MeOH. The extraction was filtered using a 0.2 μm PTFE syringe filter and stored at -20°C until analysis. A standard solution that contained 10 mg chlorogenic acid per 10 ml 70% MeOH was used to produce an external standard curve. In each sample chlorogenic acid was then quantified based on the standard curve. The concentration of chlorogenic acid was determined, and expressed per g leaf dry weight.

Phylogenetic analysis

We constructed a phylogenetic tree of the 37 plant species, and chrysanthemum using the program Phylomatic (Webb and Donoghue 2005), in which a taxon list is matched against a backbone 'metatree,'

returning a pruned tree of genus-level relationships. The backbone tree is based on the recent phylogenetic hypothesis of the Angiosperm Phylogeny Group (R20120829 for plants). We used the BLADJ algorithm of the Phylocom version 4.1 software package (Webb et al. 2008) to get branch lengths scaled to time, based on clade ages according to Wikström et al. (2001).

Statistical analysis

Prior to analyses, data from the two pots with the same soil inoculum replicate of the same disease treatment were averaged. Sterile soil came from the same homogenized source, and therefore these ten replicate pots were kept as 10 replicates. Before conducting analysis, data were checked for homogeneity of variance and normality was confirmed by inspection of the residuals. The overall effects of plant species-specific inocula and pathogen inoculation on chrysanthemum were analyzed using a linear mixed model. In the model, plant species-specific inocula and disease treatment were set as fixed factors, and soil replicate was set as random factor. In this analysis, sterile soil and no plant soil inocula were not included, as they are not species-specific soil inocula.

The pathogen effect was calculated for each soil replicate (including sterile soil and the no plant soil inoculum) as biomass in disease soil minus biomass in no disease soil. One-way ANOVA was used to determine the difference of pathogen effects between soils. A one sample *t*-test was then used to determine for each soil inoculum if the pathogen effect was significantly different from zero. The soil effects (including sterile soil and no plant soil) in the control treatment were compared using one-way ANOVA. *Post hoc* Dunnett tests were performed to compare each plant species-specific inoculum with sterile soil and with the no plant soil inoculum. The analyses described above were done for chrysanthemum aboveground biomass, belowground biomass, leaf chlorogenic acid and root/shoot ratio (Figure S2.1). Plant proportional yellowness was not normally distributed, and thus the analyses were done slightly different. The effects of plant species-specific inocula and pathogen inoculation on chrysanthemum yellowness were analyzed using a generalized linear mixed model with binomial distribution and logit link function, with plant species-specific inocula and pathogen inoculation set as fixed factors, and soil replicate as random factor. The pathogen effect was calculated for each soil replicate (including sterile soil and no plant soil inocula) as proportion yellowness in disease soil minus that in no disease soil. One-way ANOVA was used to determine the difference of pathogen effects between soils. A one sample *t*-test was then used to determine for each soil inoculum if the pathogen effect was significantly different from zero. The soil effects (including sterile soil and no plant soil inoculum) in the control treatment were compared using a generalized linear model. *Post hoc* Dunnett tests were performed to compare each plant species-specific inoculum with sterile soil and with the no plant soil inoculum. To quantify plant–soil feedback effects of a conditioning species on chrysanthemum,

the plant–soil feedback effect was calculated as natural log of the (chrysanthemum biomass (aboveground biomass + belowground biomass) on soil conditioned by that species minus average chrysanthemum biomass on sterile soil or no plant inoculum). This calculation was done for both the control treatment and the pathogen treatment. Two-way ANOVA was used to determine the overall effects of conditioning species and disease treatment on plant–soil feedback effects. A one sample *t*-test was used to determine for each species inoculum, if the effect was significantly different from zero.

To compare functional groups of the conditioning plant species (grass, forb, or legume), linear mixed models were used with plant functional group and pathogen inoculation as fixed factors, and soil replicate nested in plant species identity as a random factor, so that each conditioning species was considered a replicate. In this analysis, the sterile soil and no plant soil inoculum were not included, as these treatments were not allocated to a specific plant functional group. *Post hoc* tests were conducted with the functions 'glht' (multcomp package) and 'lsm' (lsmean package) to assess pairwise comparisons between plant functional groups. The analyses described above were done for chrysanthemum aboveground biomass, belowground biomass, root/shoot ratio and leaf chlorogenic acid. For plant yellowness, a generalized linear mixed model was used (binomial distribution and logit link function), with plant functional group and pathogen inoculation as fixed factors, and soil replicate nested in plant species identity as random factor. The same *post hoc* tests were done for pairwise comparisons of different plant functional groups.

Linear regression analysis was used to test the relationship between the phylogenetic distance of the conditioning plant species to chrysanthemum, and chrysanthemum biomass (aboveground biomass + belowground biomass). Linear regression analysis was also used to determine the relationship between chrysanthemum leaf chlorogenic acid and chrysanthemum aboveground biomass for the control and disease treatment separately. All analyses were performed in R (version 3.0.1, R Development Core Team, 2013).

Results

Above- and belowground biomass of chrysanthemum plants differed significantly between inocula and average root and shoot biomass varied more than threefold (Figure 2.1 and Table 2.2). In the control treatment, aboveground biomass of chrysanthemum grown with soil inocula from 8 species (*Thymus pulegioides*, *Crepis capillaris*, *Tagetes minuta*, *Hypochaeris radicata*, *Centaurea jacea*, *Medicago sativa*, *Vicia Sativa*, and *Trifolium arvense*) was significant lower than that of chrysanthemum grown in

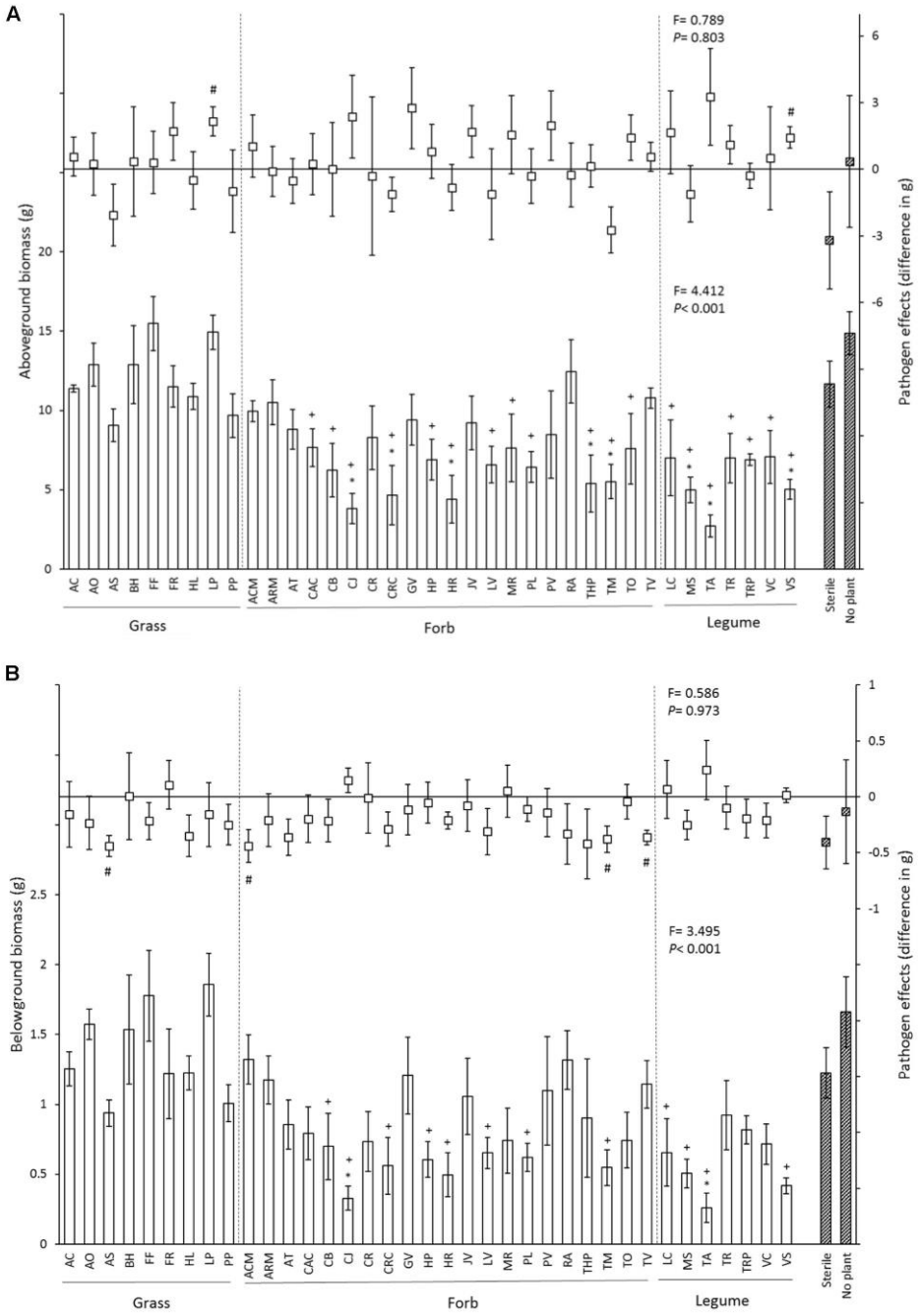

Figure 2.1. Effects of 37 species-specific soil inocula, no plant inoculum and sterile soil on chrysanthemum aboveground biomass (A) and belowground biomass (B). In each figure, bars represent chrysanthemum biomass (mean ± SE) of soil inocula in control soil, and squares represent the pathogen effect on plant biomass (biomass in *P. ultimum* soil – biomass in non-*Pythium* inoculated soil). Striped bars indicate controls. "*" Represents significant difference from the sterile soil ($P < 0.05$). "+" Represents significant difference from the no plant soil inoculum ($P < 0.05$), "#" represents significantly different from zero ($P < 0.05$). Dashed lines separate soil inocula into different functional groups. Species abbreviations are given in Table 2.1. Statistics presented in the lower part of each panel represent the effects of soil on chrysanthemum biomass in control soil, and statistics presented in the upper part of each panel indicate the effects of soil inocula on the disease severity of chrysanthemum biomass.

sterile soil. Compared to the no plant inoculum this was observed for 19 of the 37 species-specific soil inocula (Figure 2.1A). Overall, pathogen addition did not significantly influence plant aboveground biomass, and did not modify the effects of the different soil inocula on chrysanthemum aboveground biomass (no interaction between disease treatment and soil inoculum, Table 2.2). However, chrysanthemum growing with soil inocula conditioned by *Lolium perenne* and *Vicia sativa* had significantly higher aboveground biomass with *P. ultimum* than without *P. ultimum* addition (Figure 2.1A).

Root biomass of chrysanthemum grown with inocula conditioned by *Centaurea jacea* and *Trifolium arvense* was significantly lower than that of plants grown in 100% sterile soil in the no-disease treatment (Figure 2.1B). Addition of 12 species-specific soil inocula resulted in lower chrysanthemum root biomass than no plant soil inoculum. Addition of *P. ultimum* caused a significant reduction in root biomass but the interaction between disease addition and soil inoculation was not significant (Table 2.2). Addition of *P. ultimum* in soil inoculated with *Agrostis stolonifera*, *Achillea millefolium*, *Tanacetum vulgare*, or *Tagetes minuta* soil resulted in a significant reduction in root biomass. Root/shoot ratios were significantly lower in soil with *P. ultimum* addition (Figure S2.1) and the effects of *P. ultimum* addition differed between inocula resulting in a significant interaction between these two factors (Table 2.2). Grass species had neutral to positive plant–soil feedback effects on chrysanthemum, while forb and legume species had neutral to negative plant–soil feedback effects compared to sterile soil with or without *Pythium* addition (Figure S2.2A). Most plant species had negative plant–soil feedback effects on chrysanthemum when compared with the no plant inoculum either with or without *Pythium* addition (Figure S2.2B).

Table 2.2. Overall effects of identity and functional group of the conditioning plant species, and of *Pythium* addition on aboveground biomass, belowground biomass, root/shoot ratio, proportion of yellow leaves and leaf chlorogenic acid concentrations in chrysanthemum.

	df	Aboveground biomass	Belowground biomass	Root/shoot ratio	Yellowness	Chlorogenic acid
Species	36, 148	6.01***	5.18***	1.94**	1.62*	2.05**
Pythium	1, 148	2.83	23.83***	115.15***	0.13	5.87*
Species × *Pythium*	36, 148	0.74	0.66	1.73*	1.40	1.47
Functional group	2, 34	14.30***	15.46***	5.89**	6.52**	2.71
Pythium	1, 182	2.97	25.57***	103.83***	0.01	5.20*
Functional group × *Pythium*	2, 182	0.53	1.20	3.86*	0.02	1.57

*Data presented are degrees of freedom (df) and F-values from the linear mixed models and generalized linear mixed model (only used for yellowness). Asterisks indicate significant effects at ***P < 0.001, **P < 0.01, *P < 0.05.*

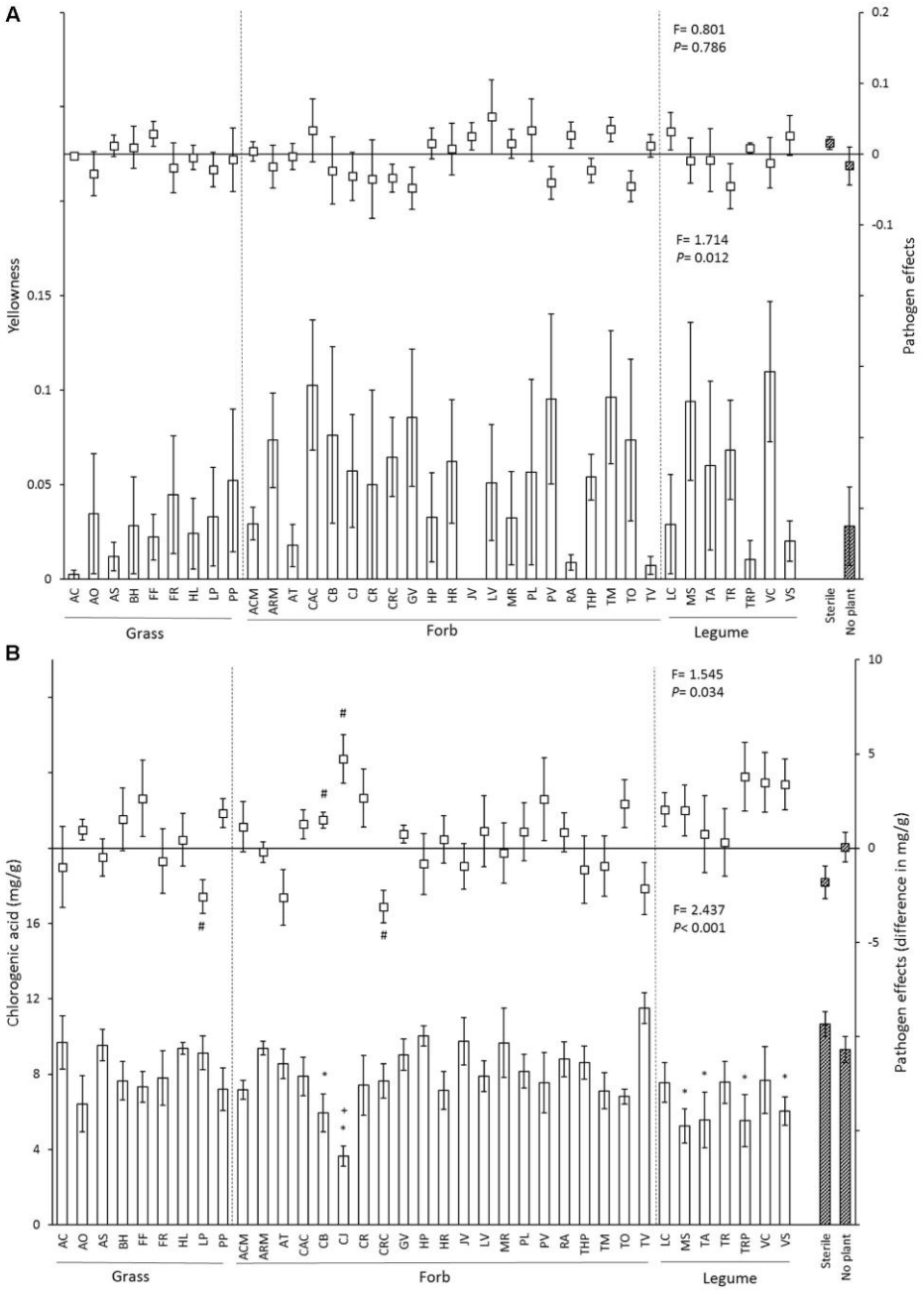

Figure 2.2. Effects of 37 species-specific soil inocula, no plant inoculum and sterile soil on chrysanthemum yellowness (A) and leaf chlorogenic acid concentration (B). In each figure, bars represent the mean (±SE) of each soil inoculum in control soil, and squares represent the pathogen effect (value in *P. ultimum* soil – value in non-*Pythium* inoculated soil). Striped bars indicate controls. "*" Represents significant difference from the sterile soil ($P < 0.05$). "+" Represents significant difference from the no plant soil inoculum ($P < 0.05$), "#" represents significantly different from zero ($P < 0.05$). Dashed lines separate soil inocula into different functional groups. Statistics presented in the lower part of each panel represent the effects of soil in control soil, and statistics presented in the upper part of each panel indicate the effects of soil inocula on the disease severity of chrysanthemum biomass.

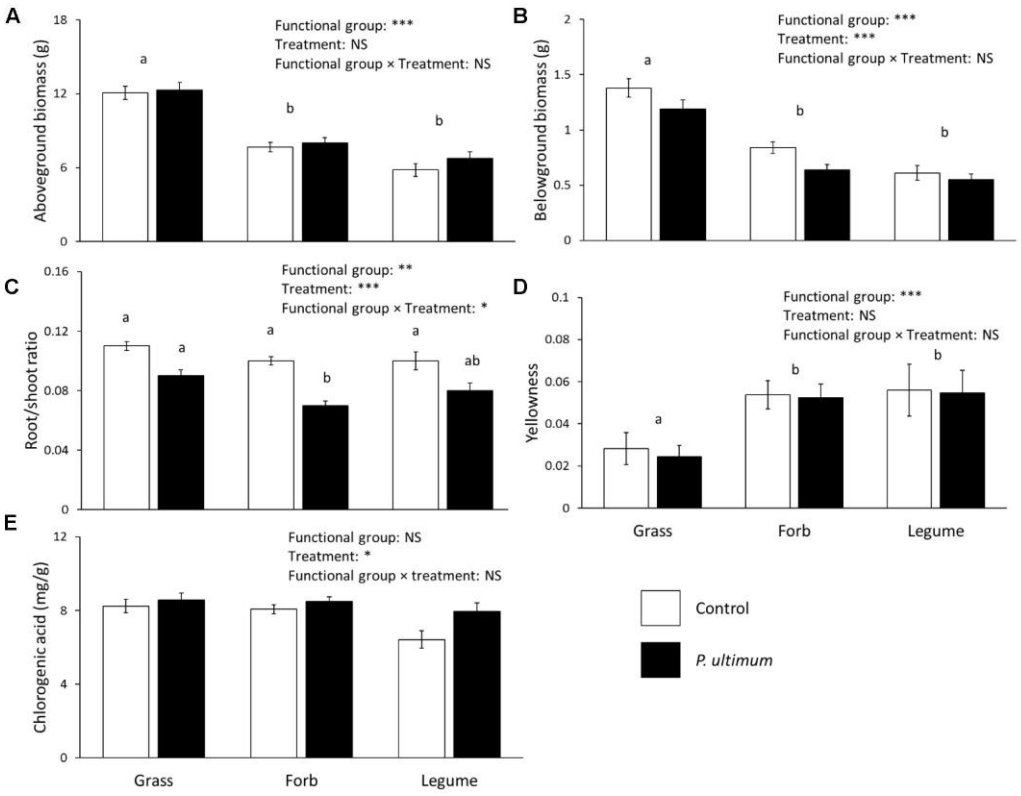

Figure 2.3. Effects of plant functional group and pathogen addition on chrysanthemum aboveground biomass (A), belowground biomass (B), root/shoot ratio (C), proportion of yellow leaves (D), and leaf chlorogenic acid concentration (E). Data show means ± SE, with white bars representing control soil, and black bars representing the *P. ultimum* treatment. Different letters indicate significant differences between functional groups ($P < 0.05$). For root/shoot ratio, different letters above bars indicate significant differences ($P < 0.05$). Full statistics are listed in Table 2.2.

The proportion of yellow leaves differed significantly between soil inocula (Figure 2.2A and Table 2.2). In the control treatment, leaf chlorogenic acid concentrations of plants growing in soils with *Capsella bursa-pastoris*, *Centaurea jacea*, *Medicago sativa*, *Trifolium arvense*, *Trifolium pratense*, and *Vicia sativa* inocula were significantly lower than in sterile soil, and leaf chlorogenic acid concentrations in soil conditioned by *Centaurea jacea* was significantly lower than no plant soil (Figure 2.2B). With *P. ultimum* inoculation, leaf chlorogenic acid concentrations of plants growing in soils with *Lolium perenne* and *Crepis capillaris* inocula were significantly lower than those in control treatment, while leaf chlorogenic acid concentrations of plants growing in soil conditioned by *Capsella bursa-pastoris*, *Centaurea jacea* were significantly higher than those growing in control soil (Figure 2.2B).

Both aboveground and belowground biomass of chrysanthemum differed significantly between functional groups of the conditioning plant species (Figures 2.3A,B). Addition of soil inocula created

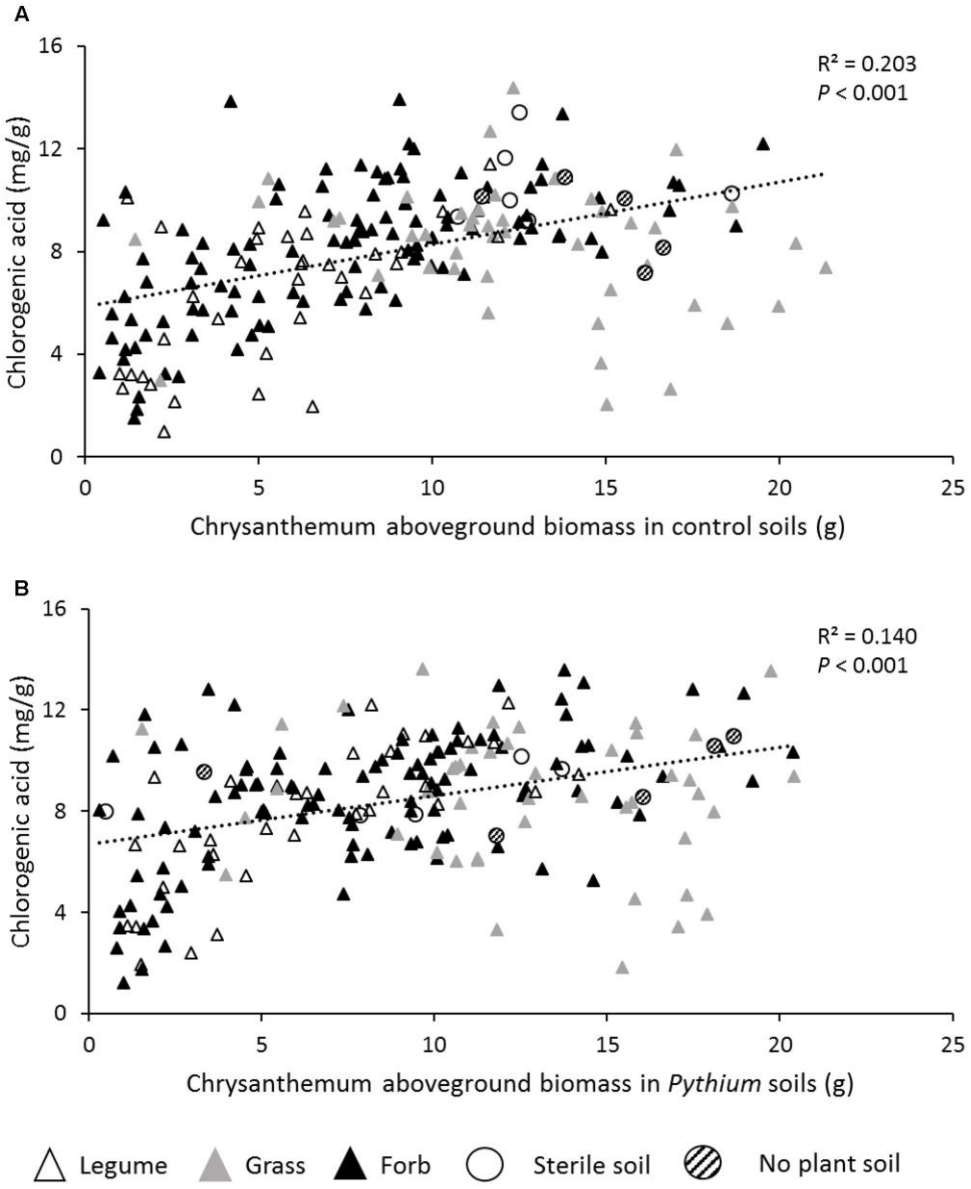

Figure 2.4. Relationships between chrysanthemum leaf chlorogenic acid concentration and aboveground biomass in control soil (A), and *Pythium* added soils (B). Black triangles represent forb inocula; Gray triangles represent grass inocula; White triangles represent legume inocula; White circles represent 100% sterile soil; Striped circles represent no plant soil.

by grasses resulted in significantly higher above- and belowground biomass of chrysanthemum than addition of forb or legume inocula. The root/shoot ratio differed between functional groups of the conditioning plant species and disease treatment, there were interactions between functional groups and the disease treatment (Figure 2.3C and Table 2.2). Root/shoot ratios did not differ between grass, legume or forb inocula in control soil but in presence of *P. ultimum*, root/shoot ratios were significantly lower with forb than with grass inocula (Figure 2.3C).

The proportion of yellow leaves differed significantly between functional groups of the conditioning plant species (Figure 2.3D). *Pythium ultimum* inoculation did not significantly influence chrysanthemum yellowness. Addition of soil inocula created by grasses resulted in significantly lower chrysanthemum yellowness than addition of forb or legume inocula.

The concentration of chlorogenic acid was significantly influenced by the identity of the plant species that was used to create the inoculum but did not differ between plant functional groups (Figure 2.3E and Table 2.2). The concentration of chlorogenic acid significantly increased in response to *P. ultimum* addition (Figure 2.3E and Table 2.2). Chlorogenic acid concentrations were positively related with chrysanthemum aboveground biomass in both the no-disease and disease treatments (Figures 2.4A,B).

There was no significant relationship between phylogenetic distance and the effect of the inoculum on chrysanthemum growth (R^2= 0.05, P = 0.11) (Figure 2.5). Topology of the phylogenetic tree is given in Supplementary Figure 2.3.

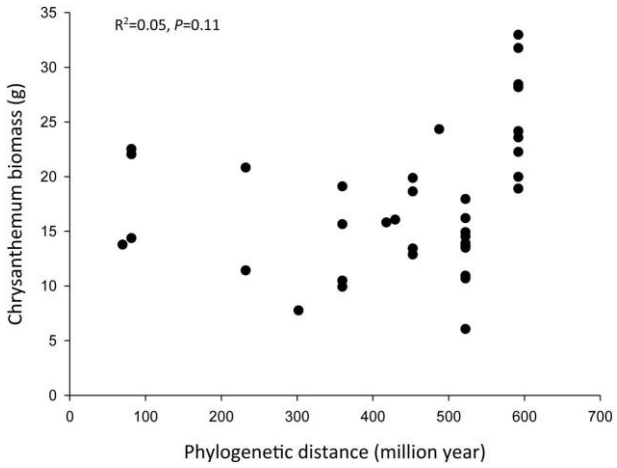

Figure 2.5. Effects of phylogenetic relationships on the chrysanthemum biomass (aboveground biomass + belowground biomass). The phylogenetic distance is the distance of conditioning plant species to chrysanthemum. The phylogenetic relationship is based on a backbone tree of the recent phylogenetic hypothesis of the Angiosperm Phylogeny Group (R20120829 for plants). We used the BLADJ algorithm of the Phylocom to get branch lengths scaled to time, based on clade ages according to Wikström et al. (2001).

Discussion

Our study shows that the identity of the plant species that conditioned the soil had a large effect on the plant–soil feedback effects on chrysanthemum growth and that plant functional group is a strong determinant of plant–soil feedback effects. When quantifying plant–soil feedback effects relative to sterile soil, most legume and forb species had negative plant–soil feedback effects on chrysanthemum biomass. In contrast, grass species had neutral to positive feedback effects on chrysanthemum biomass, and this became more apparent when *Pythium* was added. Moreover, addition of grass inocula led to more biomass and less yellowness than addition of legume or forb inocula, and led to less strong *Pythium* effects than addition of forb inocula. Importantly, and contrary to our initial hypothesis, addition of soil inocula that were created by legumes did not result in positive effects on chrysanthemum growth and did not reduce disease severity.

Inoculation with eight of the 37 soil inocula we tested negatively influenced chrysanthemum biomass compared with growth on sterile soil. Interestingly, plants grown with *Lolium perenne* inoculum that were exposed to *P. ultimum* had higher aboveground biomass than plants without *P. ultimum*. *Lolium perenne* has a highly diverse soil microbial community (Wardle et al. 2003; Clayton et al. 2005), and this species has been reported to cause increases in the density of bacteria that produce biocontrol compounds, such as 2,4-diacetylphloroglucinol, pyrrolnitrin and hydrogen cyanide (Latz et al. 2015). Thus, chrysanthemum plants grown with *Lolium perenne* inoculum may have been primed by these rhizobacteria, so that later when exposed to *P. ultimum*, the plants could respond better and faster to pathogen invasion (Pieterse et al. 2014). Pathogen infection can also lead to higher root colonization of beneficial bacteria (Rudrappa et al. 2008; Liu et al. 2014). This may explain why the biomass of chrysanthemum grown with *Lolium perenne* inoculum was larger in presence of *P. ultimum* than without the pathogen.

Chrysanthemum grown in soil with grass inocula sustained higher above- and belowground biomass than plants grown with inocula conditioned by legumes or forbs. This is partially in line with our hypothesis that grass and legume inocula have a more positive influence on chrysanthemum growth than forb inocula. Other studies with the same and with different soils have shown that the composition of the microbial community of grass-conditioned soil differs distinctly from legume-conditioned soil (Chen et al. 2008; Kos et al. 2015). Several other studies have shown that grasses in particular increase the abundance of soil bacteria, such as *Bacillus*, *Pseudomonas* and *Actinomyces*, which can act as antagonists of soil pathogens (Latz et al. 2012, 2016; Chen et al. 2016). Moreover, grasses can also increase the abundance of AM-fungi (De Deyn et al. 2010). These mechanisms may explain the better effects of grass inocula relative to legume or forb inocula in our study. Grass inocula also sustained

lower chrysanthemum yellowness than forb or legume inocula, and grass inocula overall increased plant growth and health more than legume or forb inocula. Steaming soil can kill both beneficial and pathogenic microbes in the soil, and this can lead to the rapid build-up of soil pathogens. Although grass-conditioned soil inocula did not enhance chrysanthemum growth more than that of plants grown in sterile soil, our study shows that it can provide other benefits to plants, *e.g.*, higher resistance to pathogen infection. For example, in presence of *Pythium*, addition *Lolium perenne* inoculum, resulted in higher chrysanthemum aboveground biomass. Further studies concerning the microbial interactions between soil pathogen addition and species-specific soil inocula are needed to unravel the mechanism behind this.

Surprisingly and in contrast to our hypothesis, chrysanthemum performance was worse overall with legume inocula. Legumes are often used in crop rotation to increase nitrogen content of soils (Drinkwater et al. 1998). Since in our experiments chrysanthemum plants were heavily fertilized, a nitrogen-mediated benefit of legume soil is unlikely. In contrast, the negative influence of soil inocula conditioned by legumes on chrysanthemum growth could be explained by the negative effects of legumes on certain beneficial soil bacteria (Latz et al. 2012, 2015). Legumes produce steroid saponins that act as antifungal and antibacterial compounds (Mahato et al. 1982). Moreover, the rhizobia have similar colonization strategies to both legume and non-legume plants, however, rhizobia refine their strategy to symbiosis when interacting with legumes (Soto et al. 2006, 2009). Thus, for the non-leguminous plant chrysanthemum, rhizobia would act like pathogens, explaining the reduction of plant growth in soils conditioned by legumes. Addition of soil inocula created by forbs overall also significantly decreased chrysanthemum growth. Chrysanthemum root/shoot ratios indicated plant susceptibility to *Pythium*, as *Pythium* infection reduces the root system and leads to root rot (Agrios 2005). There were no significant differences between chrysanthemum root/shoot ratios in grass, forb or legume inocula without *P. ultimum* addition. However, with *P. ultimum* addition, chrysanthemum root/shoot ratios of plants growing with in forb inocula decreased significantly more than that of plants growing with grass inocula, suggesting poor plant resistance to *P. ultimum* attack when grown with forb inocula. Forbs generally allocate less carbon to roots and have overall less microbial activity and abundance in roots than grasses (Warembourg et al. 2003; Chen et al. 2016). Hence, we speculate that the microbial community of soil inocula from forbs was smaller or less active or diverse than the microbial community of grasses. Whether this is true remains to be tested.

Plant–soil feedback effects can also be due to the modification of abiotic conditions (Ehrenfeld et al. 2005). However, in our study, we inoculated 90% homogenized sterile soil with 10% conditioned soil, and thus we minimized the heterogeneity of abiotic factors (Kardol et al. 2006). More importantly, in

the feedback phase, plants received a high dose of Hoagland fertilizer following common practice in commercial chrysanthemum greenhouses. Thus it is highly unlikely that inocula-related differences in nutrient availability influenced the results in our study, and therefore we can assume that the different plant–soil feedback effects were due to differences in microbial communities. Nutrient-rich substrates are typically exploited by r-strategist species such as *P. ultimum*, and the suppression of *P. ultimum* can be difficult in soils with high nutrient levels (van Bruggen and Semenov 2000). This may explain why the inocula were relative ineffective in suppressing *P. ultimum* infection.

Overall, the concentration of chlorogenic acid in chrysanthemum leaves differed significantly between the inocula. However, although the concentration of leaf chlorogenic acid was positively related with aboveground plant biomass, and grass inocula sustained significantly higher chrysanthemum aboveground biomass compared to forb inocula or legume inocula, the concentration of chlorogenic acid in grass inocula did not differ from those in legume inocula or forb inocula. The concentration of leaf chlorogenic acid was found to be positively correlated with plant carbon assimilation rates in sorghum (Turner et al. 2016). In our study, the levels of aboveground chlorogenic acid also increased with pathogen attack belowground compared to uninfected plants. Soil pathogens can increase aboveground plant defense even in absence of aboveground plant antagonists (Bezemer and van Dam 2005). In chrysanthemum, chlorogenic acid is related to resistance against thrips (Leiss et al. 2009, 2011), as well as to other herbivores, such as leafminers and spider mites (Kos et al. 2014). Our work therefore suggests that soil inoculation but also the presence of soil pathogens can influence the resistance of chrysanthemum against aboveground herbivorous pests and that plant–soil feedback effects may influence pest severity and biocontrol in chrysanthemum cultivations.

In contrast to our hypothesis, the plant–soil feedback effect of species closely related to chrysanthemum was not more severe than that of distantly related species. It may be possible that beyond a certain threshold phylogenetic distance, effects do become apparent, as shown by the grass clade, which is the most distantly related one. To prove this, future studies should select species across large phylogenetic scales to test their plant–soil feedback effects. Our result is in line with an increasing number of studies with wild plant species showing that phylogenetic distance is a poor predictor of plant–soil feedback effects (Pavoine et al. 2013; Kelly et al. 2014; Mehrabi and Tuck 2015; Mehrabi et al. 2015). Thus, although our study demonstrated species specific plant–soil feedback effects, these patterns may not correspond to mechanisms like shared pathogens or symbionts. Moreover, there is a growing awareness that the phylogenetic distance is a weak predictor of the dissimilarity of plant functional traits (Mouquet et al. 2012; Pavoine et al. 2013; Kelly et al. 2014). If for example, traits responsible for resource use or host susceptibility to natural enemies are not conserved, the plant species will influence or respond to

the soil in a very different way even though they are closely related (Mehrabi and Tuck 2015). Several recent studies have shown that PSF effects can be predicted from life history forms or plant traits such as root thickness or density or plant growth rate (Baxendale et al. 2014; Cortois et al. 2016; De Deyn 2017). Therefor, plant traits instead of phylogenetic distance could be a good predictor of plant–soil feedback effects.

Conclusion

In summary, we demonstrate that plant species through changes in the soil can influence the growth, disease susceptibility and the concentration of aboveground defense compounds of cultivated crop species, all in a species-specific manner. Our results further show clearly that these plant–soil feedback effects depend on plant functional groups of the species where the inocula are created from, with the highest chrysanthemum performance in soil with grass inocula. Our study with a cultivated plant species highlights that species-specific plant–soil feedback effects can also play an important role in deciphering interactions between plants and pathogens or herbivorous insects in horticulture. Disentangling the mechanisms of enhanced plant performance, and evaluating the consequences for plant yield in a real horticultural setting may allow us to implement the concept of plant–soil feedbacks in current greenhouse horticulture.

Acknowledgments

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Supplementary material

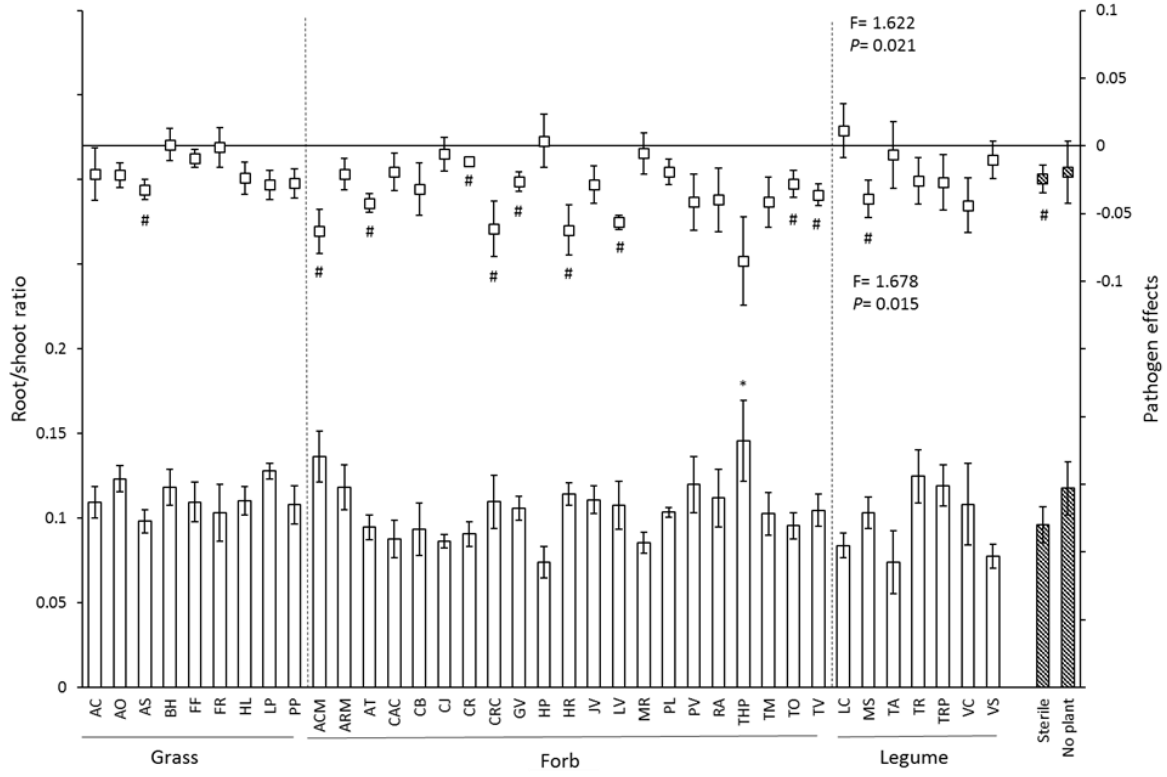

Figure S2.1. Effects of 37 species-specific soil inocula, no plant inocula and sterile soil on chrysanthemum root/shoot ratio. In each figure, bars represent chrysanthemum biomass (mean + SE) of soil inocula in control soil, and squares represent the pathogen effect on plant biomass (root/shoot ratio in *P. ultimum* soil – root/shoot ratio in non-*Pythium* soil). Stripe bars indicate controls. "*" represents significant difference from the sterile soil ($P < 0.05$). "+" represents significant difference from the no plant soil inocula ($P < 0.05$), "#" represents significantly different from zero ($P < 0.05$). Dashed lines separate soil inocula into different functional groups. Species abbreviations are given in Table 2.1. Statistics presented in the lower part of each pannel represent the effects of soil on chrysanthemum root/shoot ratio in control soil, and statistics presented in the upper part of each pannel indicate the effects of soil inocula on the disease severity of chrysanthemum root/shoot ratio.

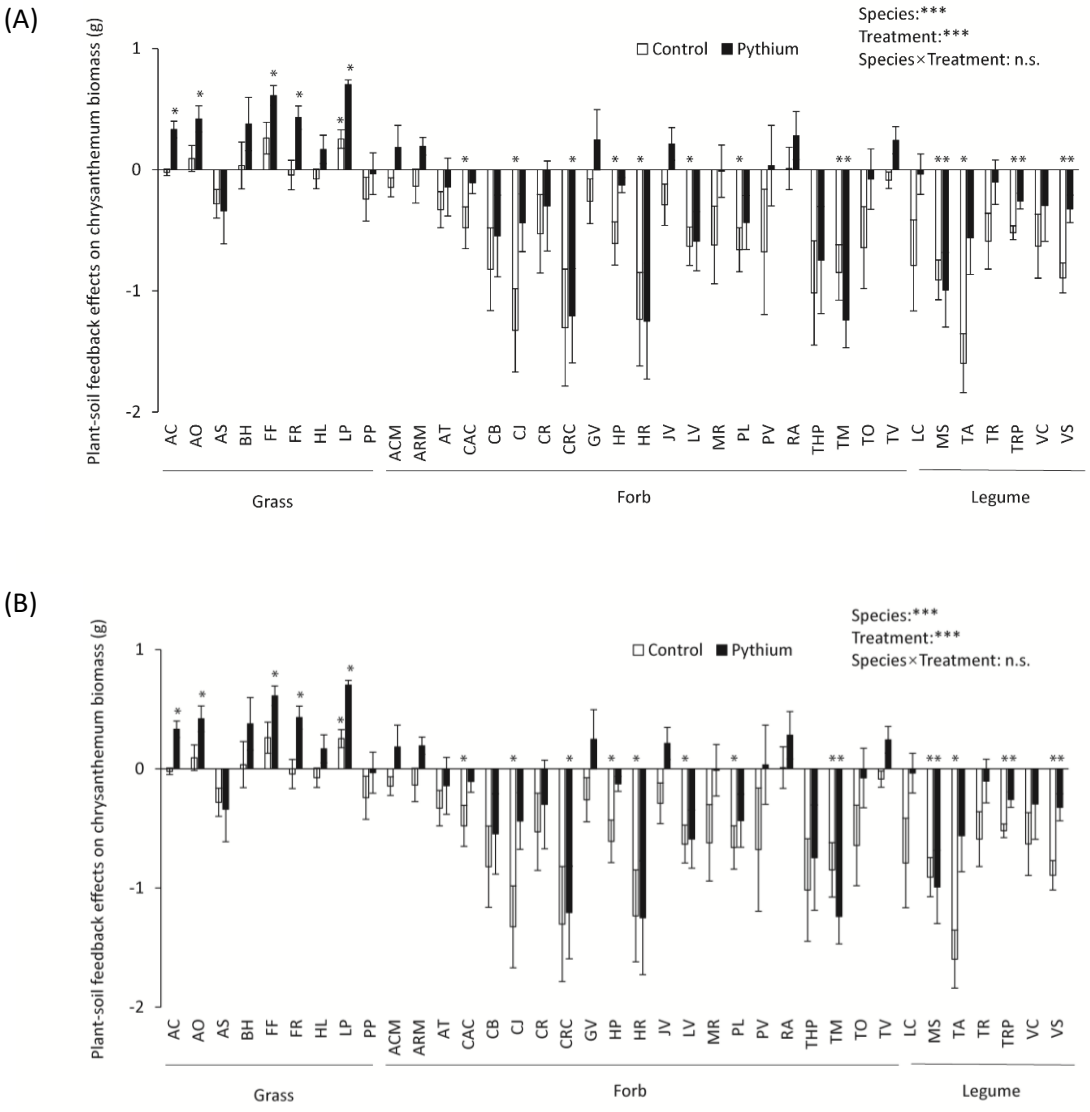

Figure S2.2. Plant–soil feedback effects of 37 plant species on chrysanthemum biomass (mean + SE). Chrysanthemum biomass calculated as the sum of aboveground biomass and belowground biomass. (A) The plant–soil feedback effect of soil conditioning by a species on chrysanthemum biomass was calculated as the natural logarithm of chrysanthemum biomass on soil conditioned by that species minus the natural logarithm of average chrysanthemum biomass on 100% sterile soil. (B) The plant–soil feedback effect of soil conditioning by a species on chrysanthemum biomass was calculated as the natural logarithm of chrysanthemum biomass on soil conditioned by that species minus the natural logarithm of average chrysanthemum biomass on no plant soil. White bars indicate control treatment; black bars indicate *P. ultimum* treatment. "*" above each bar indicates significant difference from zero ($P < 0.05$), suggesting significant difference from sterile soil. Overall effects of conditioning plant species, disease treatment, and the interaction between the two from a two-way ANOVA are present on the graph. "*" indicates significant effects. "n.s." indicates no significant effects. Species abbreviations are given in Table 2.1.

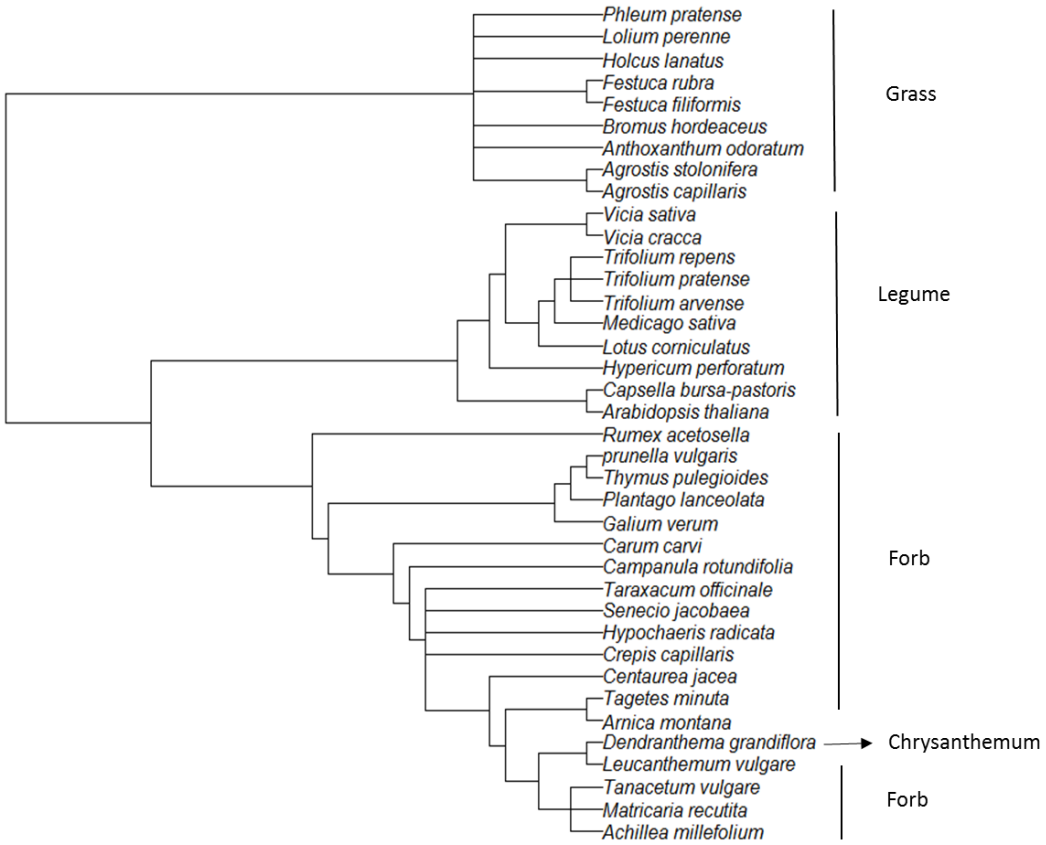

Figure S2.3. Phylogenetic relationships between conditioned plant species and chrysanthemum. Topology from Phylomatic program (Webb and Donoghue 2005).

Table S2.1. The amount of detected leaf phenolics (mean + SE of mg/g of dry leaf weight) in different soils with disease and control treatment.

		P1	P2	P3	P4	P5	P6	P7
AC	C	13.8±2.1	0.79±0.11	12.9±1.5	4.3±0.5	1.7±0.5	0.10±0.0	0.60±0.2
AC	P	12.4±2.0	0.75±0.10	13.5±1.9	3.6±0.9	2.3±0.8	0.12±0.0	0.85±0.2
ACM	C	8.9±0.5	0.54±0.03	8.4±0.3	3.3±0.3	1.9±0.2	0.11±0.0	0.81±0.1
ACM	P	13.3±0.8	0.87±0.07	14.0±1.5	4.7±0.4	1.4±0.3	0.12±0.0	0.48±0.1
AO	C	10.8±0.6	0.56±0.05	9.6±1.0	3.7±0.2	1.3±0.6	0.12±0.0	0.45±0.1
AO	P	13.7±1.7	0.81±0.09	12.7±1.1	5.2±0.6	1.3±0.5	0.14±0.0	0.49±0.1
ARM	C	13.6±1.1	0.83±0.04	12.7±0.9	4.7±0.6	1.9±0.6	0.13±0.0	0.68±0.2
ARM	P	14.1±1.5	0.88±0.08	14.8±1.9	4.9±0.4	2.0±0.4	0.11±0.0	0.70±0.0
AS	C	14.7±2.0	0.91±0.08	13.9±1.6	4.9±0.4	1.1±0.3	0.12±0.0	0.42±0.1
AS	P	13.5±2.0	0.89±0.14	14.6±1.2	4.9±0.8	2.0±0.7	0.12±0.0	0.75±0.2
AT	C	14.4±2.3	0.86±0.14	15.0±2.6	4.9±0.5	1.8±0.6	0.13±0.0	0.70±0.2
AT	P	10.6±1.7	0.64±0.09	10.7±1.2	4.0±0.6	1.9±0.4	0.12±0.0	0.78±0.1
BH	C	14.1±2.5	0.78±0.15	12.6±2.6	4.5±0.6	0.9±0.2	0.13±0.0	0.33±0.0
BH	P	12.8±1.7	0.86±0.12	13.5±0.9	4.3±0.3	1.7±0.4	0.11±0.0	0.64±0.1
CAC	C	12.9±2.1	0.68±0.14	13.7±1.2	4.4±0.8	3.7±1.7	0.10±0.0	1.22±0.4
CAC	P	12.1±3.1	0.78±0.18	13.5±2.0	4.2±1.1	2.5±1.1	0.11±0.0	0.89±0.3
CB	C	11.1±1.0	0.57±0.06	9.6±0.8	3.8±0.2	2.2±0.5	0.10±0.0	0.84±0.1
CB	P	10.0±1.7	0.67±0.13	11.6±1.0	3.7±0.5	2.9±0.8	0.11±0.0	1.11±0.3
CJ	C	13.9±3.6	0.57±0.14	10.5±1.4	4.6±0.8	1.6±0.7	0.12±0.0	0.70±0.3
CJ	P	14.0±3.0	0.85±0.18	14.3±2.5	5.0±0.7	0.9±0.4	0.12±0.0	0.38±0.1
CR	C	13.0±2.2	0.74±0.21	14.6±3.3	4.1±0.5	2.1±0.7	0.10±0.0	0.75±0.2
CR	P	7.7±2.1	0.47±0.15	9.1±1.4	3.4±0.5	0.4±0.2	0.11±0.0	0.24±0.1
CRC	C	8.9±0.3	0.48±0.06	10.3±0.9	3.0±0.3	3.1±1.1	0.10±0.0	1.08±0.2
CRC	P	16.3±1.9	0.98±0.10	16.1±1.2	4.9±0.4	2.1±0.6	0.11±0.0	0.70±0.2
FF	C	15.3±2.5	0.83±0.15	12.5±2.2	4.8±0.6	1.5±0.6	0.14±0.0	0.57±0.2
FF	P	13.5±2.2	0.91±0.16	14.4±2.1	4.2±0.5	2.4±0.7	0.12±0.0	0.73±0.1
FR	C	10.9±1.5	0.58±0.05	11.0±1.4	3.5±0.4	2.4±0.9	0.10±0.0	0.84±0.3
FR	P	13.0±1.5	0.80±0.12	11.9±1.8	4.5±0.3	1.2±0.2	0.12±0.0	0.43±0.0
GV	C	12.4±1.9	0.72±0.11	11.8±1.7	3.9±0.4	2.1±0.5	0.12±0.0	0.72±0.1
GV	P	16.6±2.7	1.02±0.13	16.4±1.4	5.3±0.6	1.7±0.7	0.12±0.0	0.54±0.2
HL	C	14.3±2.5	0.80±0.13	13.2±1.4	4.7±0.8	2.1±0.3	0.12±0.0	0.80±0.1
HL	P	14.7±1.5	0.97±0.08	14.7±1.1	4.7±0.6	1.3±0.7	0.11±0.0	0.42±0.2
HP	C	16.2±0.8	0.98±0.04	14.0±0.8	5.3±0.2	1.3±0.5	0.13±0.0	0.46±0.1
HP	P	12.6±1.5	0.86±0.12	14.3±1.4	4.3±0.4	1.6±0.2	0.10±0.0	0.57±0.0

		P1	P2	P3	P4	P5	P6	P7
HR	C	13.8±0.6	0.73±0.10	12.5±0.8	4.2±0.3	1.7±0.3	0.10±0.0	0.63±0.1
HR	P	11.8±2.6	0.71±0.14	13.0±2.5	4.2±0.6	1.7±0.6	0.10±0.0	0.71±0.2
JV	C	11.5±2.0	0.72±0.13	12.4±1.6	3.8±0.4	2.2±0.8	0.11±0.0	0.79±0.2
JV	P	12.8±0.5	0.81±0.04	12.9±0.7	4.6±0.4	1.5±0.4	0.12±0.0	0.53±0.1
LC	C	11.2±0.7	0.63±0.07	11.5±0.9	4.2±0.4	1.9±0.6	0.11±0.0	0.7±0.20
LC	P	11.6±1.2	0.83±0.07	12.7±0.9	4.4±0.5	1.4±0.3	0.11±0.0	0.53±0.1
LP	C	11.9±3.6	0.62±0.16	11.8±2.2	3.2±0.4	1.9±0.5	0.09±0.0	0.60±0.1
LP	P	10.7±1.7	0.67±0.09	10.8±0.7	3.4±0.5	1.9±0.7	0.11±0.0	0.71±0.2
LV	C	9.8±0.4	0.60±0.04	9.6±0.5	3.7±0.3	1.7±0.4	0.11±0.0	0.66±0.1
LV	P	10.8±2.0	0.66±0.11	12.6±1.9	3.5±0.3	3.0±0.6	0.10±0.0	1.05±0.1
MR	C	18.5±3.3	0.99±0.18	15.3±2.1	5.9±0.7	1.0±0.4	0.12±0.0	0.32±0.1
MR	P	16.2±3.1	1.00±0.14	16.0±1.9	4.7±1.0	2.2±0.9	0.09±0.0	0.65±0.2
MS	C	8.0±1.9	0.39±0.12*	8.0±0.9	3.4±0.7	1.4±0.2	0.12±0.0	0.76±0.0
MS	P	12.6±1.1	0.76±0.07	13.2±1.1	4.9±0.5	1.3±0.5	0.12±0.0	0.53±0.2
PL	C	9.7±1.8	0.58±0.1	11.0±1.0	3.4±0.5	1.8±0.4	0.11±0.0	0.82±0.2
PL	P	10.4±1.4	0.70±0.10	12.3±1.4	3.7±0.3	2.4±0.5	0.11±0.0	0.93±0.2
PP	C	12.1±2.5	0.70±0.14	11.8±2.1	4.2±0.6	1.1±0.3	0.13±0.0	0.48±0.1
PP	P	18.9±4.3	1.15±0.19	18.0±3.9	5.1±0.7	0.9±0.3	0.11±0.0	0.29±0.1
PV	C	10.2±1.9	0.52±0.17	11.3±1.0	3.3±0.6	1.5±0.5	0.09±0.0	0.58±0.1
PV	P	13.7±1.1	0.89±0.04	14.1±0.7	5.1±0.5	1.2±0.3	0.13±0.0	0.47±0.1
RA	C	13.3±1.9	0.74±0.09	12.9±1.0	4.7±0.8	2.0±0.8	0.11±0.0	0.77±0.2
RA	P	12.2±1.8	0.83±0.13	13.0±1.7	4.0±0.5	2.3±0.8	0.11±0.0	0.81±0.3
TA	C	9.6±2.2	0.48±0.11	9.5±2.0	3.9±0.6	1.7±0.6	0.12±0.0	0.74±0.1
TA	P	9.1±1.3	0.52±0.08	10.6±1.0	4.1±0.5	1.5±0.4	0.11±0.0	0.70±0.2
THP	C	11.7±2.6	0.64±0.13	11.9±1.6	3.6±0.8	2. 6±0.6	0.10±0.0	0.97±0.2
THP	P	8.7±2.3	0.52±0.14	12.0±1.1	3.0±0.8	3.3±0.8	0.09±0.0	1.30±0.3
TM	C	10.2±1.3	0.56±0.09	8.8±1.1	3.5±0.2	2.6±0.7	0.10±0.0	0.98±0.2
TM	P	10.9±1.8	0.64±0.15	12.1±1.5	3.9±0.6	3.0±0.9	0.09±0.0	1.06±0.2
TO	C	10.1±1.2	0.52±0.07	10.1±0.9	3.7±0.3	2.9±0.8	0.11±0.0	1.08±0.2
TO	P	10.2±1.5	0.73±0.11	11.8±1.2	3.9±0.5	2.7±0.4	0.11±0.0	1.04±0.1
TR	C	15.0±2.6	0.78±0.12	13.0±1.7	4.7±0.5	0.5±0.2	0.10±0.0	0.23±0.0
TR	P	11.6±2.1	0.76±0.14	12.8±1.6	4.6±0.5	1.8±0.2	0.12±0.0	0.73±0.0
TRP	C	6.7±0.8	0.40±0.06*	7.0±0.7	2.8±0.3	1.8±0.5	0.10±0.0	0.87±0.2
TRP	P	13.6±1.8	0.85±0.08	13.4±1.2	5.2±0.4	1.7±0.4	0.12±0.0	0.66±0.1
TV	C	17.6±1.3	1.09±0.07	17.0±1.5	5.2±0.4	2.0±0.5	0.13±0.0	0.61±0.1
TV	P	11.1±1.3	0.78±0.09	12.2±1.1	3.7±0.3	2.3±0.5	0.09±0.0	0.93±0.2

		P1	P2	P3	P4	P5	P6	P7
VC	C	9.9±1.4	0.60±0.12	9.3±1.4	3.8±0.4	1.2±0.2	0.11±0.0	0.54±0.1
VC	P	10.5±1.0	0.68±0.05	11.4±1.1	4.4±0.5	2.0±0.9	0.11±0.0	0.79±0.2
VS	C	7.6±1.5	0.25±0.07**	8.9±1.1	3.2±0.5	1.8±0.3	0.13±0.0	0.94±0.1
VS	P	8.8±1.0	0.55±0.08	9.8±0.8	3.8±0.3	1.4±0.3	0.11±0.0	0.62±0.1
Sterile	C	12.9±1.1	0.91±0.07	12.7±0.9	4.3±0.3	1.4±0.4	0.11±0.0	0.52±0.1
Sterile	P	11.5±2.2	0.67±0.17	13.6±0.7	4.0±1.0	4.8±2.6	0.10±0.0	0.78±0.1
No plant	C	12.0±1.7	0.66±0.10	15.1±3.1	3.4±0.5	3.8±1.4	0.09±0.0	1.14±0.3
No plant	P	17.5±2.3	1.01±0.09	17.0±2.3	5.0±0.5	1.8±0.6	0.11±0.0	0.49±0.1

P: *P. ultimum* treatment; C: control treatment; P1 to P7 are detected unidentified leaf phenolics. Asterisks indicate significant difference from sterile soil. ****P*<0.001, ***P*<0.01, **P*<0.05.

Chapter 3

Synergistic and antagonistic effects of mixing monospecific soils on plant-soil feedbacks

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Abstract

Background and aims Plants influence the soil they grow in, and this can alter the performance of other, later growing plants in the same soil. This is called plant-soil feedback and is usually tested with monospecific soils, *i.e.* soils that are conditioned by one plant species. Here, we test if plant-soil feedbacks of inocula consisting of mixtures of monospecific soils can be predicted from the effects of the component inocula.

Methods Chrysanthemum plants were grown in sterile soil inoculated with eight monospecific conditioned soils and with mixtures consisting of all pairwise combinations. Plant biomass and leaf yellowness were measured and the additivity was calculated.

Results On average, plant biomass in the mixed inocula was slightly but significantly (6%) lower than predicted. In contrast, when growing in mixed inocula, plants showed 38% less disease symptoms than predicted. Moreover, the larger the difference between the effects of the two monospecific soils on plant growth, the higher the observed effect in the mixture exceeded the predicted effects.

Conclusions We show that mixed monospecific soils interact antagonistically in terms of plant growth, but synergistically for disease symptoms. Our study further advances our understanding of plant-soil feedbacks, and suggests that mixing soils can be a powerful tool to steer soil microbiomes to improve plant-soil feedback effects.

Key words: Plant-soil feedback, Plant health, Additivity, Interaction, Species-specific soil

Introduction

Plants are an important determinant of the composition of soil communities, and the effect of a plant on the soil microbial community can subsequently affect the performance of other plants that grow later in that soil, a phenomenon termed plant-soil feedback (van der Putten et al. 2013; Bever et al. 1997). Such plant-soil feedback effects are typically recorded as the net outcome of all negative and positive effects on plant growth. However, a single plant can increase the density of soil organisms with both negative (*e.g.* soil pathogens) and positive (*e.g.* beneficial soil organisms such as plant growth promoting bacteria) effects (Mendes et al. 2013; Raaijmakers et al. 2009). An important question that has received little attention is how mixing soils conditioned by different plant species, each with positive and negative effects, influences the net effect of this soil on plant performance.

When mixing soils or in fact any two characteristics, three possible effects can be expected: synergistic, additive, or antagonistic. First, the outcome of mixing two specific soil communities can be stronger than the two individual effects together (synergistic effect). For example, Hendriks et al. (2013) found that when the same amount of soil was added, mixtures of soil collected from different monocultures sustained higher plant biomass than pure monoculture soils. On the contrary, mixing soil communities could also lead to antagonistic effects, so that the mixed effects are weaker than what would be predicted from the individual effects. Several studies reported, for example, that combinations of biocontrol microbial strains fail to reduce specific plant diseases, even though the individual strains all have suppressing effects on the disease, suggesting that antagonistic interactions occur among these microbial strains (Schisler et al. 1997; Sarma et al. 2015). Third, it is also possible that positive and negative interactions between plants and soil organisms counterbalance each other, so that the mixed soil effect is simply the sum of individual effects (additive effects; Singh et al. 2015). Ladygina et al. (2010), for example, showed that when added in isolation, arbuscular mycorrhizal fungi increased plant community productivity, while addition of soil decomposers decreased productivity, and addition of root herbivores had no effect. When these three groups of soil organisms were added together, their effect on productivity could be predicted from adding up the individual negative and positive effects. Due to the potential for interactions between soil microorganisms, whether plant performance in mixed soil communities can be predicted from the plant performance in the soils conditioned by a single plant species (*i.e.* monospecific soils), is an open question.

Whether plant-soil feedback effects in mixtures of monospecific soils, are additive, synergistic or antagonistic may depend on how different the effects of the monospecific soils are, but to our knowledge, there are no studies yet that have tested how the difference between two monospecific soils influences the effects on plant growth or plant health. However, from plant competition experiments it is known

that synergistic effects occur more often when characteristics of the two species that compete differ considerably. Growing together two species that occupy different niches, allows the species to capture resources in ways that are complementary, leading to aboveground overyielding (Mommer et al. 2010; Cardinale et al. 2007). Similarly, decomposition experiments have shown that mixing plant species-specific litters that differ greatly in chemistry leads to higher than expected decomposition rates, but this is not true when the different litters are relatively similar in chemical composition (Harguindeguy et al. 2008). Thus, when mixing two factors (*e.g.* two plant species-specific litters) that greatly differ in composition or effect, the net effect of the mixture tends to be better than predicted. Hence, we may also expect that mixing two monospecific soils with distinctly different soil communities, and thus with largely different effects on plant growth should result in a more positive effect of plant growth than what is predicted based on the sum of the effects of the individual soil communities.

In this study we examine how mixing soils conditioned by different plant species influences net plant-soil feedback effects on plant growth and leaf yellowness (a plant health indicator) (Reddy 2016). In a previous study, we tested the plant-soil feedback effects of 37 different plant species and observed that inoculation of soil conditioned by several species led to increased growth and resistance against *Pythium*, while inoculation of soils conditioned by other species reduced growth and resistance (Ma et al. 2017). In the current study, we selected eight plant species (that previously showed positive and negative soil effects on chrysanthemum growth) and examined the effects of mixing these plant species-specific soil inocula on chrysanthemum performance. Specifically, we ask: (i) can the effects of mixed soil inocula be predicted from the effects observed with the monospecific soil inocula that are used for the mixture? (ii) is such effect synergistic, additive, or antagonistic? and, (iii) how is this related to the absolute difference between the effect of the two monospecific inocula? For each inoculum we also examined how its effect is influenced by mixing it with other inocula.

Materials and methods

Plant material

The focal plant in our study is *Dendranthema X grandiflora* (Ramat.) Kitam. cv. Grand Pink (Chrysanthemum, syn. *Chrysanthemum X morifolium* (Ramat.) Hemsl., Asteraceae). Chrysanthemum is one of the major cut flower crops that is cultivated in soil in glasshouses. The soil is sterilized regularly by steaming to control soil pathogens (Thuerig et al. 2009; Tamm et al. 2010). Hence, in this system the use of inoculating conditioned soil inocula into sterilized bulk soil represents a realistic scenario. Chrysanthemum cuttings were provided by the breeding company FIDES by Dümmen Orange (De Lier, The Netherlands).

Experimental set-up

The experiment consisted of two phases, in the first phase, the conditioning phase, we grew eight plant species in monocultures to create monospecific soils. In the second phase, the test phase, we used mixtures of all combinations of two monospecific soils (including mixtures of two identical monospecific soils), and used these soils as inocula to test the effects on chrysanthemum growth.

Phase I: Conditioning phase

For the conditioning phase, soil was collected (5–20 cm deep) in June 2015 from a former arable field, which has become a natural grassland since 1996 (Mossel, Ede, The Netherlands). The sandy-loam soil was homogenized and sieved (1 cm mesh size) to remove coarse fragments and all macro-arthropods. Pots (13 × 13 × 13 cm) were filled with a homogenized mixture of field soil and sterilized field soil in a 1:1 ratio. The sterilized soil was added to minimize potential differences in soil nutrients and to provide a niche for the soil microbes to grow and hence increase the potential for plantspecies-specific effects on the soil community. Pots were filled with 1.6 Kg of soil (based on dry weight). Soil sterilization was done by gamma irradiation (> 25 K Gray gamma irradiation, Isotron, Ede, The Netherlands).

Eight plant species were used to condition the soils: *Anthoxanthum odoratum* (AO), *Bromus hordeaceus* (BH), *Festuca filiformis* (FF), *Lolium perenne* (LP), *Holcus lanatus* (HL), *Rumex acetosella* (RA), *Galium verum* (GV) and *Hypochaeris radicata* (HR). Seeds of all species were obtained from a wild plant seed supplier (Cruydt-Hoeck, Assen, The Netherlands). Seeds were surface sterilized in 3% sodium hypochlorite solution for 1 min, rinsed and germinated on sterile glass beads in a climate chamber at 20 °C (16 h/8 h, light/dark).

Five one-week-old seedlings were transplanted in monocultures in each pot, and there were ten replicate pots for each species. In total, the conditioning phase comprised of 80 pots (monocultures of 8 plant species × 10 replicates). Seedlings that died during the first week of the experiment were replaced. As a few seedlings died later, after two weeks, the number of seedlings in each pot was reduced to four so that the density was the same in all pots. All pots were placed randomly in a greenhouse with 70% RH, 16 h 21° (day) and 8 h 16° (night). Natural daylight was supplemented by 400 W metal halide lamps (225 μmol $s^{-1}m^{-2}$ photosynthetically active radiation, one lamp per 1.5 m^2). The pots were watered by hand every other day. Ten weeks after transplanting, the plants were carefully removed from each pot and the largest roots were removed from the soil as they may act as a source for re-growing plants. Finer roots were left in the soil as the rhizosphere around these roots may include a major part of the microbial

rhizosphere community. The soil from each pot was homogenized and stored separately in a plastic bag at 4 °C until used in the test phase so that there were 10 replicate soils for each plant species. The soils are called “soil inocula” hereafter.

Phase II: Test phase

For the test phase, the conditioned soil from the first phase was used as inoculum. There were two types of inocula, monospecific inocula (*i.e.* soil conditioned by one plant species), and heterospecific soil inocula (*i.e.* 1:1 mixtures of two monospecific conditioned soils). Mixtures of all combinations were used, thus the feedback phase comprised of 360 pots (28 combinations of mixed inocula × 10 replicates + 8 conspecific mixtures × 10 replicates). Pots of 1 L (11 × 11 × 12 cm; length × wide × height) were filled with a homogenized mixture of 10% inoculum and 90% sterile field soil (see above). Two 5 cm chrysanthemum cuttings (without roots) were planted in each pot. Prior to planting, the soil in each pot was well watered and 100 ml half-strength Hoagland nutrient solution was added (Li and Cheng 2015). The pots were randomly placed on trolleys, each trolley had 48 pots and was tightly covered with a thin transparent plastic film for 10 days to create a closed environment with high humidity that favors rooting. After 10 days, most of the cuttings had rooted. Non-rooted cuttings were removed and from pots where both cuttings had rooted, a randomly selected chrysanthemum cutting was removed. Plants were fertilized following grower practice: half-strength Hoagland nutrient solution (0.9 mS/cm electric conductivity) for the first two weeks, and full strength Hoagland solution (1.4 mS/cm electric conductivity) during the following two weeks. For the last two weeks, the strength was increased to 1.6 mS/cm electric conductivity. The density of pots on each trolley was reduced two weeks after the beginning of the second phase to 32 pots per trolley so that there was 10 cm space between each pot. All pots were randomly arranged in a greenhouse compartment kept under the same conditions as described for the conditioning phase.

Plant performance

Eight weeks after planting the cuttings, all plants were harvested. For each plant, the number of leaves that showed yellowness and the total number of leaves were recorded. Leaf yellowness in chrysanthemum is symptomatic for diseases such as those caused by soil pathogens like *Verticillium* and *Fusarium* (Reddy 2016). The characterization of yellowness was based on observations by eye, and for all leaves which were characterized as yellow, an area of at least 5% of the leaf was yellow. Yellowness was then calculated as the proportion of yellow leaves (number of yellow leaves relative to the total number of leaves on that plant). Plants were clipped at soil level and roots were washed over a

sieve (2 mm mesh). Shoot and root biomass was then oven-dried (60 °C for 3 days) and weighed. Plant biomass was calculated as the sum of plant shoot and root dry weight.

Calculations and statistical analysis

The predicted (additive) effects of mixed inoculum (*e.g.* combination AB) on chrysanthemum biomass and yellowness were calculated as (effect of inoculum A + effect of inoculum B)/2. This was done for each soil replicate separately. Then, the observed effects of mixed inocula were compared with their predicted effects. If there is no significant difference between these two effects, this indicates that the effects of mixing are additive. A significantly lower than predicted effect indicates antagonistic interactions, while a significant higher effect indicates synergistic interactions. In this analysis, we used each mixture as a replicate. For this, we averaged the values of the replicate samples of each mixture. A paired *t*-test was used to test if the observed effects of mixing inocula (real values) were significantly different from the predicted effects. This analysis was done for chrysanthemum biomass and yellowness. For the statistical analysis, chrysanthemum yellowness was arcsine-transformed, as yellowness was entered as proportional data. The average effect for all inocula combinations is presented in the main text. The detailed results for each mixture (*i.e.* each combination of two monospecific soils) are presented in the supplementary materials (Fig. S3.1).

To examine whether there was a relationship between the difference among two monospecific inocula on chrysanthemum performance and the difference between the observed and predicted effects when mixing these two inocula, we used linear regression. We first calculated the absolute difference between the effects of the two monospecific inocula, and this was plotted against the difference between the observed and the predicted effect of the mixture. In this latter calculation, positive or negative values indicate synergistic or antagonistic interactions between component monospecific inocula respectively. Data were checked for homogeneity of variance and normality by inspection of the residuals before the analysis. We then determined the sign and strength of the linear relationship between these two parameters.

To examine for each conditioning species the effects of mixing on plant biomass and leaf yellowness, we compared the eight inocula that contained each conditioning species using a one-way ANOVA. Individual comparisons were based on a post-hoc Tukey test. The response of each monospecific inoculum to mixing was determined by comparing the effects of the heterospecific mixtures containing a monospecific inoculum to the effect of the monospecific inoculum: (response of inoculum A to mixing = the average effect of heterospecific mixtures containing inoculum A – the effect of

monospecific inoculum A). This was done for each replicate separately. A one-sample *t*-test was used to test for each inoculum if the response was significantly different from zero. Values that are not different from zero indicate that the response is not different from the monospecific mixture, values less than zero indicate that heterospecific mixing has a negative influence, while values larger than zero indicate that mixing has a positive effect. One-way ANOVA was used to determine if these mixing effects on biomass differed between inocula, and a generalized linear model was used to analyze differences in yellowness. The chrysanthemum biomass and yellowness in each mixed inoculum are listed in Table S3.1 and Table S3.2 of the supplementary materials.

To test whether there were significant differences between mixtures which contained a specific monospecific inoculum on plant biomass and yellowness, we used one-way ANOVA. A *post-hoc* Tukey test was used for pairwise comparisons between different mixed inocula. All analyses were performed in R (version 3.0.1, R Development Core Team, 2017).

Results

The biomass of plants exposed to mixed soil inocula was lower than what was predicted from the effects of the monospecific inocula, suggesting that on average two soil communities interact antagonistically with respect to plant growth. However, leaf yellowness was also lower than predicted and therefore soil mixing benefited plant health (Fig. 3.1). With regard to each monospecific inoculum, for four out of eight plant species, observed chrysanthemum biomass was significantly lower in mixtures than predicted. For two out of eight species leaf yellowness was significantly lower in mixtures than predicted (Fig. S3.1).

For total plant biomass, there was a weak but significantly positive relationship between the absolute difference among the two monospecific inocula and how much the observed effects of their mixture varied from the predicted effects (Fig. 3.2a). This means that the larger the difference between the effects of the two monospecific soils on plant growth is, the higher the observed effect of the mixture exceeds the predicted effect. The difference between observed and predicted yellowness became more negative with increasing differences between the effects of the two component inocula (Fig. 3.2b). This relationship was not driven by the inoculum with most extreme effects, *H. radicata*, since removal of this species-species soil inoculum from the analysis did not alter the trend (Fig. S3.2).

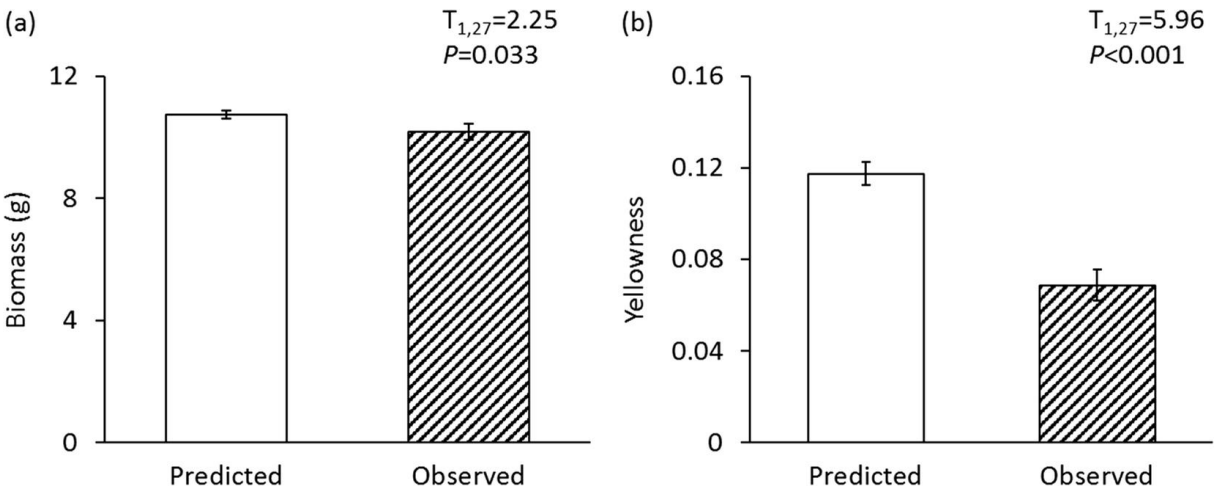

Fig. 3.1 Mean (± SE) predicted (white bars) and observed (hatched bars) effects of soil mixing on chrysanthemum biomass (a) and yellowness (b). White bars represent predicted effects of mixed inocula based on effects in component monospecific inocula (effect of inoculum A + effect of inoculum B)/2. T and *P* values from a paired *t*-test are also presented. The figure shows the average effects of all mixtures. The effects for each separate two-species soil mixture are presented in Fig. S3.1.

Overall, chrysanthemum biomass differed significantly among monospecific inocula. Greatest gain in chrysanthemum biomass was observed when grown with monospecific *A. odoratum* inoculum, and lowest with *H. radicata* inoculum (Fig. 3.3a). On average, plant biomass in heterospecific mixtures was significantly lower than in conspecific mixtures for inocula that included soil conditioned by *A. odoratum*, *B. hordeaceus* and *L. perenne*. Mixing soil conditioned by *H. radicata*, the most negative monospecific inoculum, with other inocula resulted in more biomass than when chrysanthemum was grown in monospecific soil conditioned by *H. radicata* (Fig. 3.3a). Leaf yellowness did not differ between monospecific inocula. Yellowness in heterospecific mixtures did not significantly differ from those in conspecific mixtures, except for soil conditioned by *H. radicata*, where heterospecific mixing resulted in lower levels of leaf yellowness (Fig. 3.3b).

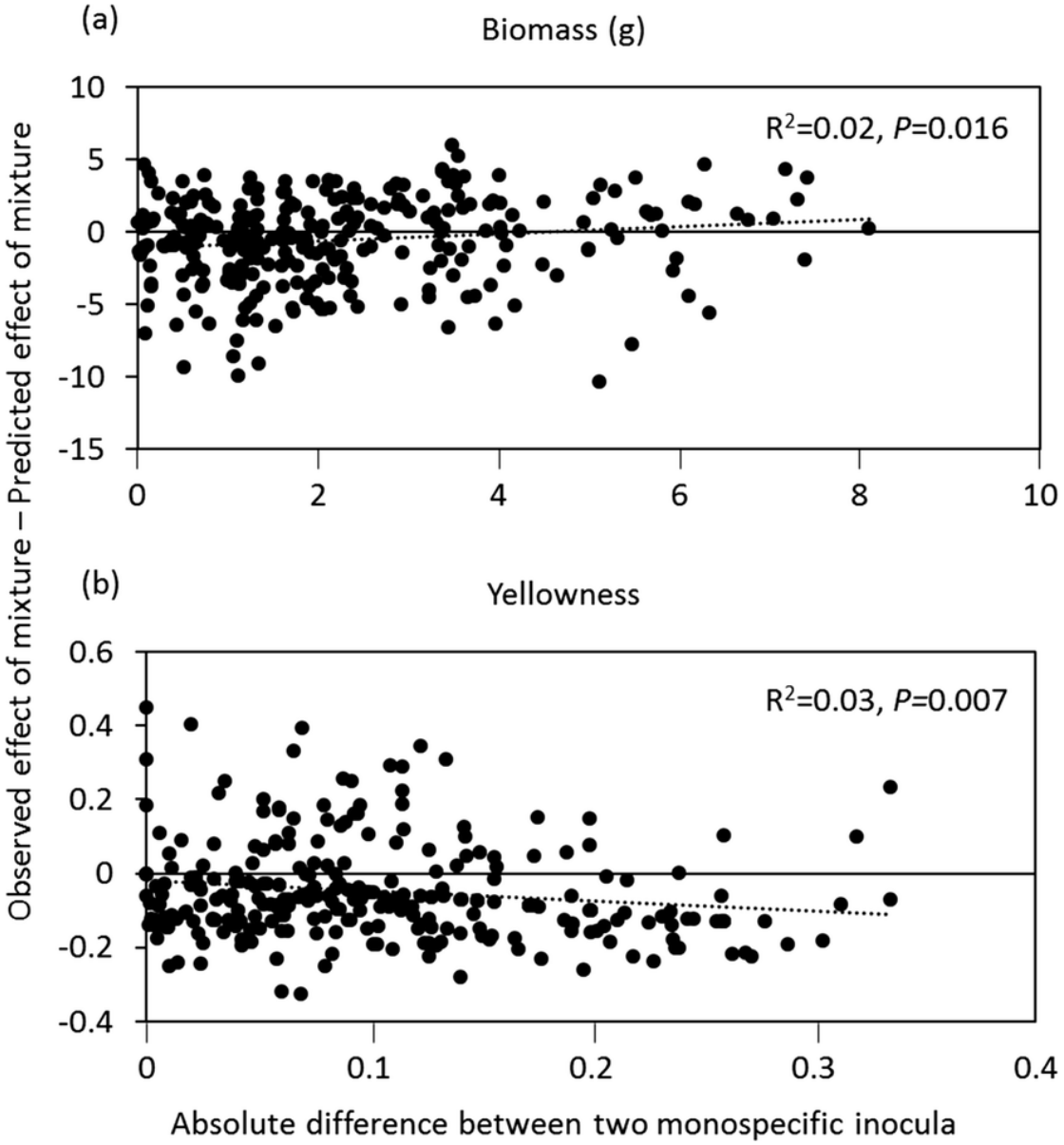

Fig. 3.2 Relationship between the difference among two monospecific inocula on plant biomass (a) and yellowness (b), and the difference between the observed and predicted effects when mixing these two inocula. The difference of monoculture inocula is calculated as (|effect of inoculum A – effect of inoculum B|). The difference between observed and predicted effects of the mixtures is calculated as (observed value of mixture A + B – predicted value of mixture A + B). The goodness of fit (R^2) and *P* value of both regressions are also presented.

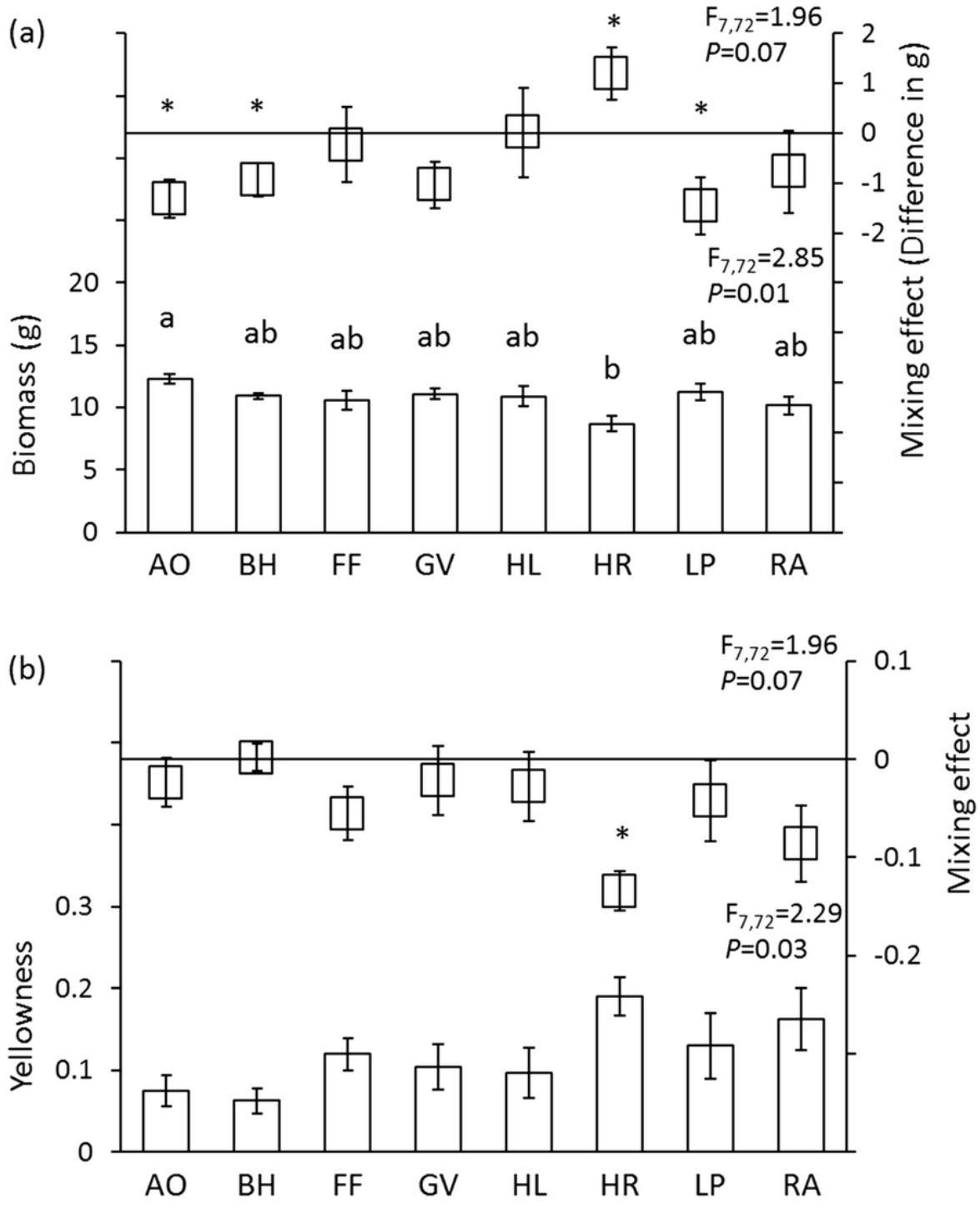

Fig. 3.3 Effects of monospecific soil inocula on chrysanthemum biomass (a), and yellowness (b) in conspecific and heterospecific mixtures. Mixing effects are calculated as (average effects of heterospecific mixtures that include inoculum A – effects of monospecific inoculum A). The zero line indicates that mixing does not differ from the effects of the monoculture species inocula. "*" represents significantly different from zero (one-sample *t*-test, $P < 0.05$). The bars represent the effects of each monospecific inoculum (mean ± SE). F and *P* values from a one-way ANOVA are also presented. Bars with identical letters are not significantly different from each other based on a *post hoc* Tukey test. Species abbreviations are explained in the Materials and methods section.

When inocula conditioned by *A. odoratum*, *B. hordeaceus*, *F. filiformis* and *H. lanatus* were mixed with other inocula, this did not lead to differences between these mixtures on plant biomass. In contrast, mixing inocula conditioned by *R. acetosella*, *H. radicata*, *G. verum* or *L. perenne* with other inocula resulted in significant differences between these mixtures on plant biomass, as mixing of *L. perenne* with inocula conditioned by forbs resulted in lower biomass (Table S3.1). Leaf yellowness did not differ in these comparisons (Table S3.2).

Discussion

Our study shows that plant-soil feedback effects that arise from mixing monospecific conditioned soils are on average non-additive. In this experiment, the biomass and the yellowness of plants growing in pots with mixed inocula were significantly lower than what was predicted from the effects of the monospecific inocula. Moreover, when the difference in the effects between two monospecific inocula increased, the effects on plant biomass and health when mixing these two inocula became weakly positive than expected. This suggests that synergistic interactions in soil microbial communities increase when the effects of the two monospecific inocula are more different, implying that the synergistic or antagonistic effects of soils on plant growth can be predicted based on the difference between their individual effects.

Plant biomass was not enhanced by mixing plant monospecific soils, but leaf yellowness was reduced. The observed reduction in plant biomass and leaf yellowness relative to the effects predicted from the monospecific soils could be due to several reasons. First, the mixed inocula consisted of 50% of both monospecific soils, and as such only consisted of 50% of the density of soil microorganisms of both monospecific soils. Previous studies found that a reduction in volume of a soil inoculum reduces the effect of the inoculum on the plant (St-Denis et al. 2017; Mendes et al. 2011; Hol et al. 2017). However, whether the relative reduction of the effectiveness of the soil inoculum is linearly or non-linearly related to the change in soil volume is unknown. Our results suggest that when the volume of one monospecific soil in the inoculum is reduced by 50%, the effects of the soil microorganisms on plant growth are reduced by more than 50%, as the mixed inocula had weaker effects than what was predicted. Thus, the observed reduction in plant biomass and leaf yellowness may be due to the weakened effects of beneficial or detrimental microbes in mixed inocula. Second, mixed soils most likely harbor a higher microbial diversity than monoculture soils, and this may increase the likelihood of introducing in the mixture both detrimental and beneficial organisms that will interact with the plant. However, the observed chrysanthemum leaf yellowness which is presumably caused by soil pathogens was also reduced, and this indicates that soil pathogens are not the reason of the reduction in plant biomass in mixed inocula. Instead, it is possible that enhanced plant health may be at the cost of plant growth as

interacting with beneficial soil microbes can be costly for plants (Morgan et al. 2005). However, such interaction can also provide extra functions such as disease suppression or induced resistance (Pieterse et al. 2014; Mendes et al. 2011), as we observed in terms of leaf yellowness. It is important to note that, in this study, we only recorded plant performance during one growth cycle and that the soil-mediated effects reported here may become stronger during subsequent plant growth cycles when the soil community has developed further.

The fact that mixing monospecific soils leads to non-additive effects on plant growth is in line with other studies that reported non-additive effects of mixing soils from different origins on plant growth (Brandt et al. 2013; Burns et al. 2017). Brandt et al. (2013) found that plants grew worse in homogenized mixtures of soils that are of different origins than what would be predicted from the effects observed in plants grown in monospecific soil. Later, Burns et al. (2017) showed that the composition of soil microbial communities in soil mixtures differs from that in monoculture soils. They proposed that the influence of the microbial community on plants could be either via direct effects of soil microbes on the plant or via indirect effects of soil microbes on soil nutrient availability for the plant. In their study, the pots contained 100% live soil. In contrast, in our study we inoculated 90% sterile soil with 10% live conditioned soil to homogenize abiotic conditions (Kardol et al. 2006). Furthermore, in our experiment, all soils received high levels of fertilization, following farmer's practices further minimizing differences in abiotic conditions (*i.e.* nutrient levels). Therefore we suggest that the mixing effects that we observed on plant performance were likely to be caused by interactions between soil microbes (Brinkman et al. 2010). In the studies by Brandt et al. (2013) and Burns et al. (2017), the effects in homogenized soil mixtures were compared with effects in heterogeneous monospecific soil, and differences in patchiness between different soil treatments may have an important impact on the results (Wubs and Bezemer 2016). In our study, we compared the homogenized soil mixtures with homogenized monospecific soils, thus narrowing down the number of factors that could potentially influence the results. To our knowledge, no study has tested both responses in terms of plant growth and plant health to soil mixing. The leaf yellowness results in our study provide important information about the negative influence that certain soils can have on plant health, and how such negative effects can be reduced by mixing soils.

Our results show that there was a weak relationship between the magnitude of the difference between the effects of two monospecific soil inocula on plant growth and how much the observed effect differed from the predicted effect. This trend did not change when we excluded mixtures that contained soil conditioned by *Hypochaeris radicata* from the analysis, the soil inoculum that had the most negative influence on chrysanthemum performance. This result has three implications, first, when mixing two monospecific soils with similar positive effects on plant growth, the effect of the mixture will be worse

than the sum of their individual effects. Similarly, mixing two monospecific soils with similar negative effects will not reduce the negative effect more than what would predicted from the monospecific soil effects. Third, when mixing two soils that have opposing effects, the effect of the mixture tends to be more positive than the sum of their individual effects.

The effects discussed above are on plant growth, with regard to yellowness, there were only additive to synergistic interactions (in terms of plant benefits). Our results therefore suggest that mixing two inocula will alleviate negative effects of monospecific inocula on plant health. Mixing two soils with different microbial communities (and we expect with large differences in their effect on plant growth) can lead to synergistic effects if adding a second soil will be complementary to the existing microbial community. This is in accordance with studies about mixing plant species or plant litters, which have found that synergistic interactions are likely to happen when the two species or litters have very different characteristics (Mommer et al. 2010; Cardinale et al. 2007; Harguindeguy et al. 2008; Gartner and Cardon 2004). Further studies should examine the differences in soil microbial composition before and after mixing.

In summary, this study demonstrates that the plant-soil feedback effects of monospecific conditioned soils are non-additive when mixed. On average, plants show less disease symptoms but also grow worse in soil with mixed inocula compared with prediction. Moreover, with increasing differences among the effects of two soil inocula on plant growth and health, the synergistic effects also increase when the soils are mixed. The synergistic and antagonistic effects of soils are two extreme outcomes in the wide range of potential interactions that can occur. We created an antagonistic to synergistic continuum and such continuum could provide important information about predicting the effect of mixing two soils on plant. For example, if our results can be extended to other systems, we may be able to select soil inocula that vary greatly in how they affect plant growth and mix them, in order to create synergistic interactions. This study therefore exemplifies how soil microbiomes can be manipulated to enhance disease resistance (Pineda et al. 2017). Our study with the cut flower chrysanthemum also highlights the role and potential of using plant-soil feedbacks in influencing the health and yield of a horticultural crops (Dias et al. 2015; Pineda et al. 2017).

Acknowledgements

The authors thank Manuela van Leeuwen (Dümmen Orange) for providing chrysanthemum cuttings, Rene Corsten (Delphy) for advice on optimal growing conditions of chrysanthemum. We thank Chinese Scholarship Council (CSC) for funding HM, and the Netherlands Organization for Scientific Research (NWO, project no. 870.15.080) for funding this research. This is publication 6537 of the Netherlands Institute of Ecology (NIOO-KNAW).

Supplementary material

Table S3.1 Plant biomass in each mixed inoculum (mean + SE). Each row/column combination indicates the average biomass for this inocula combination. For each column, the F value from a one-way ANOVA is also presented. *,**,*** indicate significant differences at $P<0.05$, $P<0.01$ or $P<0.001$, respectively. Within each column, values followed by identical letters are not significantly different based on a *post hoc* Tukey test. The shaded part of the table is presented twice. Plant species abbreviations are described in the material and methods section.

Inocula	AO	BH	FF	GV	HL	HR	LP	RA
AO	12.3+0.4a	10.1+0.8a	11.0+1.2a	10.9+ 0.5b	10.1+0.8a	10.5+ 0.7ab	12.9+0.3c	11.6+ 0.8b
BH	10.1+0.8a	10.9+0.3a	8.8+1.0a	10.5+ 1.2ab	10.3+0.9a	9.6+ 0.8ab	11.4+0.7c	9.2+ 0.5ab
FF	11.0+1.2a	8.8+1.0a	10.6+0.8a	10.7+ 0.6ab	12.7+0.8a	9.7+ 0.9ab	10.4+0.6bc	9.1+ 0.9ab
GV	10.9+0.5a	10.5+1.2a	10.7+0.6a	11.1+ 0.4b	10.4+0.6a	11.2+ 0.8b	7.5+1.1ab	9.1+ 0.4ab
HL	10.1+0.8a	10.3+0.9a	12.7+0.8a	10.4+ 0.6ab	10.9+0.8a	10.9+ 0.5ab	11.7+0.8c	10.1+ 1.2ab
HR	10.5+0.7a	9.6+0.8a	9.7+0.9a	11.2+ 0.8b	10.9+0.5a	8.7+ 0.6ab	7.7+0.5ab	9.7+ 1.1ab
LP	12.9+0.3a	11.4+0.7a	10.4+0.6a	7.5+ 1.1a	11.7+0.8a	7.7+ 0.5a	11.3+0.7c	7.1+ 0.9a
RA	11.6+0.8a	9.2+0.5a	9.1+0.9a	9.1+ 0.4ab	10.1+1.2a	9.7+ 1.1ab	7.1+0.9a	10.2+ 0.7ab
Mixture(df=7,72)	1.88	1.16	1.90	2.99**	1.11	2.26*	9.17***	2.23*

Table S3.2 Plant yellowness in each mixed inoculum (mean + SE). Each row/column combination indicates the average biomass for this inocula combination. For each column, the F value from a one-way ANOVA is also presented. The shaded part of the table is presented twice. Plant species abbreviations are described in the material and methods section.

Inocula	AO	BH	FF	GV	HL	HR	LP	RA
AO	0.07±0.02	0.06±0.04	0.06±0.05	0.03±0.02	0.11±0.05	0.03±0.02	0.03±0.02	0.02±0.02
BH	0.06±0.04	0.06±0.02	0.09±0.04	0.07±0.03	0.10±0.05	0.05±0.03	0.03±0.02	0.05±0.02
FF	0.06±0.05	0.09±0.04	0.12±0.02	0.09±0.03	0.02±0.02	0.07±0.04	0.07±0.03	0.05±0.03
GV	0.03±0.02	0.07±0.03	0.09±0.03	0.10±0.03	0.05±0.04	0.04±0.04	0.17±0.05	0.12±0.04
HL	0.11±0.05	0.10±0.05	0.02±0.02	0.05±0.04	0.10±0.03	0.03±0.02	0.08±0.05	0.08±0.05
HR	0.03±0.02	0.05±0.03	0.07±0.04	0.04±0.04	0.03±0.02	0.19±0.02	0.09±0.04	0.08±0.04
LP	0.03±0.02	0.03±0.02	0.07±0.03	0.17±0.05	0.08±0.05	0.09±0.04	0.13±0.04	0.13±0.04
RA	0.02±0.02	0.05±0.02	0.05±0.03	0.12±0.04	0.08±0.05	0.08±0.04	0.13±0.04	0.16±0.04
Mixture(df=7,72)	0.78	0.28	0.72	1.37	0.65	1.93	1.61	1.60

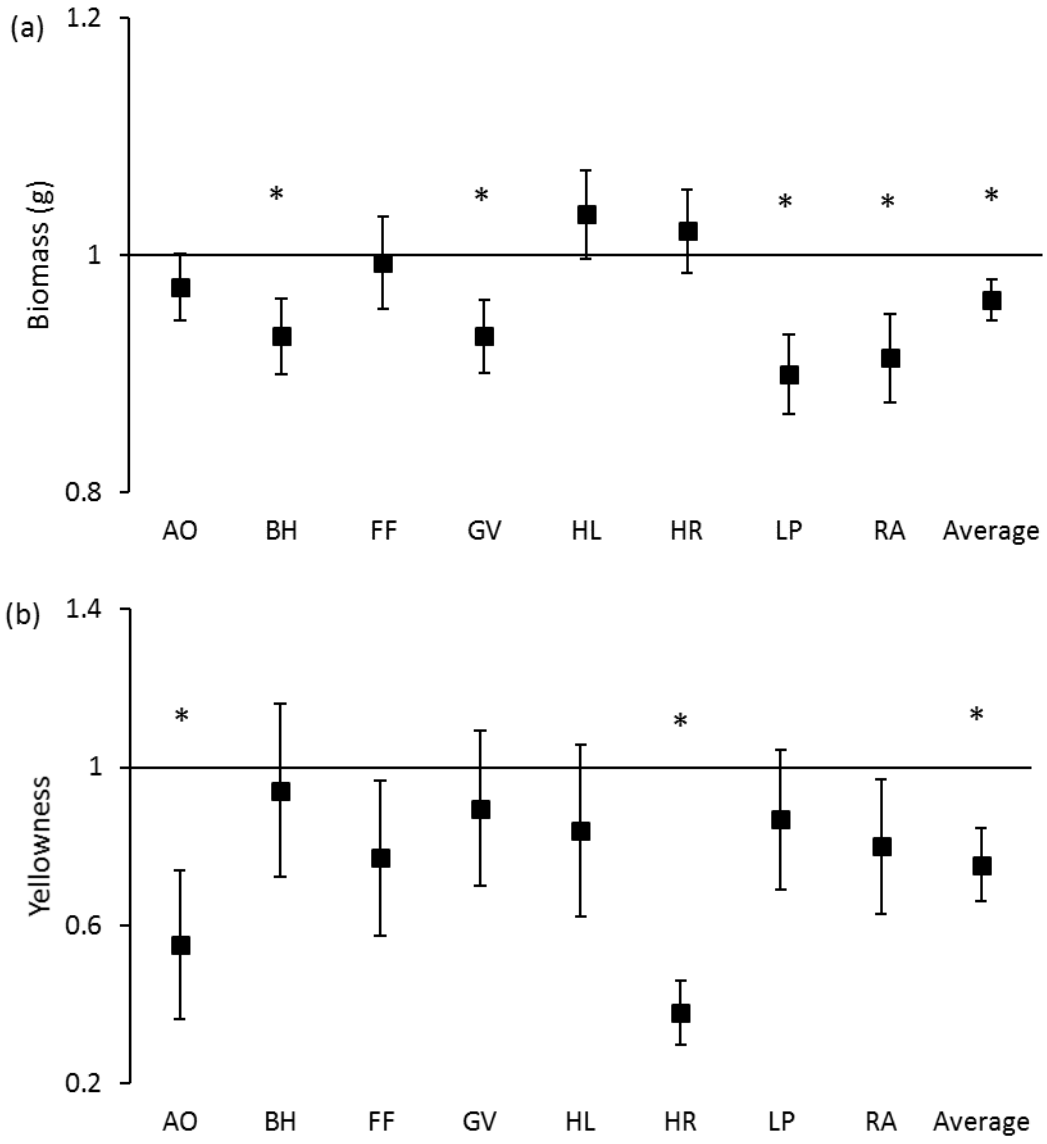

Fig.S3.1 The ratio of predicted and observed effects of mixtures on chrysanthemum biomass (a) and yellowness (b). Means are shown (+/- 1 SE). Predicted effects of mixed inocula are calculated as (effect of inoculum A + effect of inoculum B)/2. Figures show the average effects of all mixtures which contained the respective monospecific inoculum. "Average" means the average effect of all mixed inocula, which is the same as presented in Fig.3.1. "*" represents significant difference from one sample *t*-test.

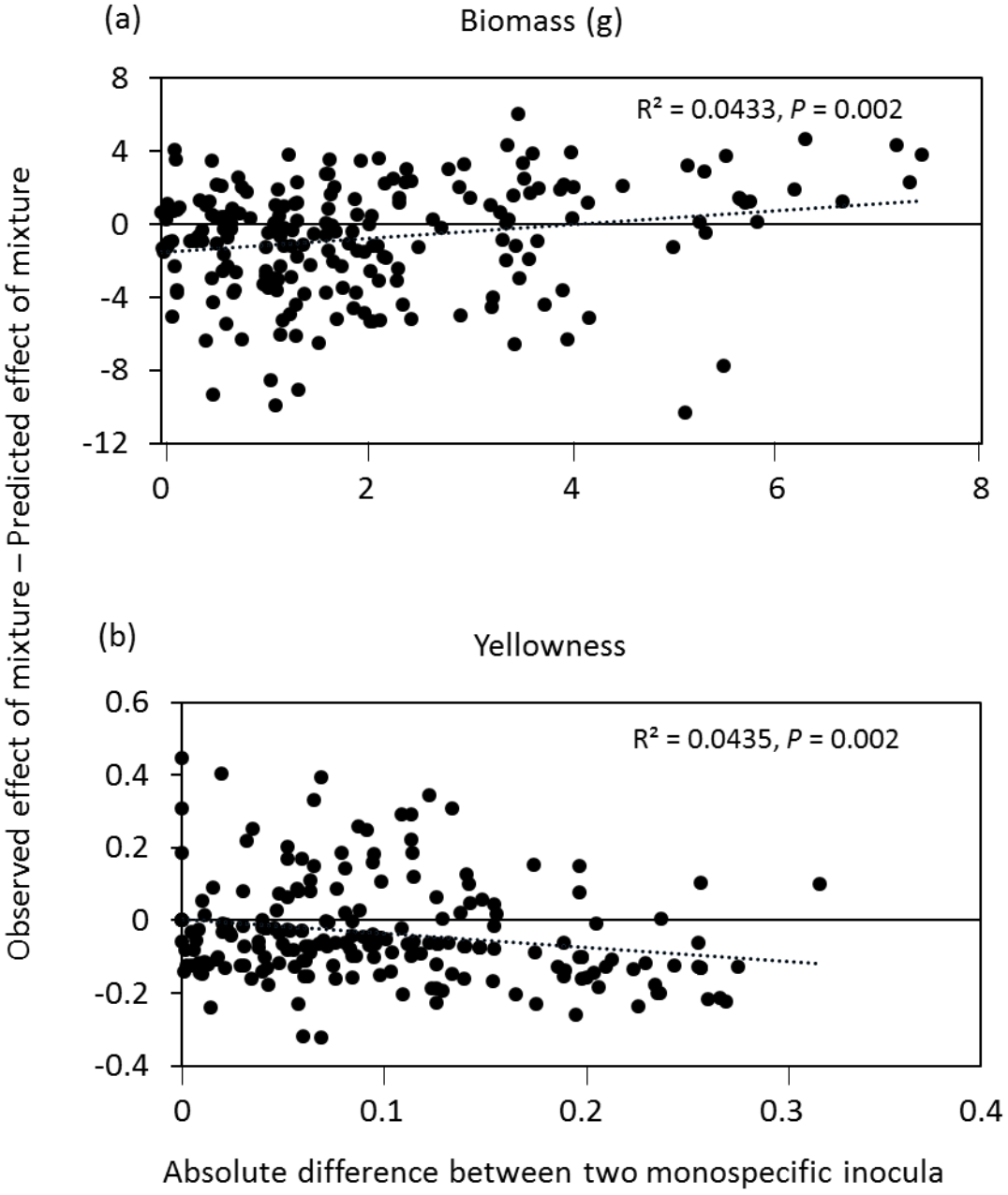

Fig.S3.2 Relationship between the difference among the effects of monospecific inocula on plant biomass (a) and yellowness (b), and the difference between the observed and predicted effects of their mixtures (excluding mixtures which containing HR and monospecific HR inocula). The difference of monoculture inocula is calculated as an absolute value |effect of inoculum A – effect of inoculum B|. The difference between observed and predicted effects of the mixtures is calculated as (observed value of mixture A+B – predicted value of mixture A+B). The fit (R^2) and *P* value of both regressions are also presented.

Chapter 4

Carry-over effects of soil inoculation on plant growth and health under sequential exposure to soil-borne diseases

Haikun Ma*, Ana Pineda, Andre W. G. van der Wurff, T. Martijn Bezemer

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Abstract

Background and aims Most plant-soil feedback and inoculation studies are limited to one growth cycle. We examined the effects of inoculation with eight plant-conditioned soils on chrysanthemum during two sequential growth cycles. The plants were also exposed sequentially to soil diseases.

Methods In cycle 1, plants were grown in sterile soil inoculated or not with plant-conditioned soils, and exposed or not to *Pythium* or root feeding nematodes. In cycle 2, new plants were grown in soil from cycle 1 or in new 100% sterile soil. Plants were exposed again to *Pythium*, or to soil with pathogens and nematodes collected from a commercial chrysanthemum greenhouse.

Results After two cycles, effects of soil inoculation on plant growth were still present. Chrysanthemum exhibited a negative conspecific feedback response, but this was less strong in inoculated soils. *Pythium* or nematode addition did not affect plant growth. However, addition of pathogen-containing soil from the commercial greenhouse reduced plant growth in sterile soil but increased growth in plant-conditioned soils.

Conclusions Inoculation with plant-conditioned soil can reduce the negative conspecific plant-soil feedback of chrysanthemum. Our study further advances our understanding of the temporal dynamics of conspecific and heterospecific plant-soil feedbacks, and how they interact with soil-borne diseases.

Key Words: Carry-over effects, Soil inoculation, Chrysanthemum, Soil-borne disease, Plant-soil feedback.

Introduction

Belowground plant pathogens, parasites, herbivores and mutualists can greatly influence the performance of plants (Wardle et al. 2004). Plants also selectively affect soil biota by releasing organic compounds into the soil via *e.g.* root exudation or dead plant material (Wardle et al. 2004). This interdependency leads to feedbacks between plants that grew first in the soil and plants that grow later in that soil, a phenomenon called plant-soil feedback (van der Putten et al. 2013; Bever et al. 1997). Many plant species grow better in soil where previously another plant species was grown than in their own soil (Kulmatiski et al. 2008; van de Voorde et al. 2011; Cortois et al. 2016). There is increasing interest to implement soil ecological concepts such as plant-soil feedbacks into agricultural systems to enhance soil health and quality and therefore the sustainability of crop production (Pineda et al. 2017; Mariotte et al. 2017; Zhou et al. 2017; Wang et al. 2017a). It is well-known that inoculation with specific soil microbes can reduce the susceptibility of a crop to pests and diseases. However, a number of studies have reported that many of these disease-suppressive strains poorly colonize and survive in the rhizosphere (*e.g.* Gómez Expósito et al. 2017; Alabouvette et al. 2009). Inoculation of soil with beneficial microbiomes rather than with individual microbial species can also transfer disease-suppressive properties, and may be more efficient than inoculation with specific microbial strains (Pineda et al. 2017; Schlatter et al. 2017; Ma et al. 2017; Chaparro et al. 2012). An important challenge is to examine how long these soil community inocula remain effective, and thus whether we can improve crop health in the longer term using heterospecific plant-soil feedback principles.

The biotic and abiotic legacies that plants leave in the soil can have long-lasting effects on other plants, and this has been detected both in natural and agricultural systems (Kulmatiski et al. 2006, Kulmatiski and Beard 2011; Detheridge et al. 2016). These long-term soil mediated effects could be due to the persistence of plant allelochemicals in the soil (Huang et al. 2013), or due to long dormancy stages of certain soil organisms (Lennon and Jones 2011), or because many free-living soil microbes can survive in the soil without their host (Lapsansky et al. 2016). Recently, two microcosm studies showed that after sequentially growing different plant species in the same soil, the first plant species, via a legacy left in the soil, still influenced a third plant species even though another plant species had been grown in the soil intermediately (Wubs and Bezemer 2018; Bezemer et al. 2018). However, the plant that grew most recently in the soil had the strongest influence, via its effect on the soil, on the last plant (Wubs and Bezemer 2018). Thus, by repeatedly growing a focal plant in soil inoculated with a microbial community, we would expect that the impact of the microbial community that was inoculated at the beginning may still be detected in later growth cycles. However, we also expect that the influence of the focal plant itself on later plant growth (*i.e.* conspecific plant-soil feedback) will increase with time. Hence, the

effect of the microbial community that was inoculated initially will diminish over time (Bezemer et al. 2018).

How carry-over effects of inoculation on plant growth are influenced by exposure of the focal plant to soil-borne diseases is unknown. Several studies have shown that repeated exposure to soil pathogens during different growth cycles affects plant growth more negatively than a single exposure to soil pathogens (Hajihassani et al. 2013; Khan and Siddiqui 2017; Whitelaw-Weckert et al. 2013). In such sequential interactions between plants and belowground pathogenic organisms, the first inoculation with a pathogen may have a stronger negative influence on plant growth than the second inoculation (Siddiqui et al. 1999; Castillo et al. 1998; Wurst and Ohgushi 2015; Pung et al. 1991). However, the severity of sequential inoculations with pathogens will also depend on the microbial community that is present. For example, microbial communities that negatively affect the growth of a focal plant may also increase the susceptibility of this plant to other soil-borne diseases, while soil microbial communities that suppress soil pathogens, may reduce the susceptibility of the focal plant to later exposure to (other) soil pathogens (Mallon et al. 2018).

In this study, we examine how inoculation with soil microbial communities from eight plant species influences the growth and disease susceptibility of chrysanthemum during two growth cycles with sequential exposure to different soil-borne diseases. The focal plant chrysanthemum (*Dendrathema X grandiflora*) is a commercial cut-flower, and in commercial greenhouses in the Netherlands, the soil is sterilized regularly by steaming to control soil pathogens (Thuerig et al. 2009; Tamm et al. 2010). The selection of the eight plant species used in this study to condition the soil was based on a previous study, in which we observed that inoculation with plant-conditioned soil had highly variable effects on chrysanthemum performance and that the effects were species-specific (Ma et al. 2017). Based on these previous results, we selected plant species with positive and negative effects on chrysanthemum, to examine how these positive and negative soil feedback effects develop when chrysanthemum is also sequentially exposed to soil-borne diseases. We exposed plants in inoculated and un-inoculated soil to the root pathogen *Pythium ultimum* and the root knot nematode *Meloidogyne incognita* in the first growth cycle. *Pythium ultimum* is an important root pathogen in chrysanthemum and causes symptoms such as root rot (Reddy 2015; Pettitt et al. 2011). *Meloidogyne incognita* is a sedentary root endoparasite, which causes root galls in chrysanthemum and subsequent leaf yellowing, eventually resulting in stunted growth (Johnson and Littrell 1970; Siddiqui et al. 2014). In the second growth cycle, we grew new chrysanthemum plants in the soil from cycle 1. These plants were exposed again to *Pythium ultimum* or to 10% “diseased soil” collected from a commercial chrysanthemum greenhouse with a severe *Meloidogyne* infestation. We tested four hypotheses: 1) the effects of inoculation at the beginning of the

first growth cycle will remain present in the second growth cycle; but 2) negative conspecific feedback effects of chrysanthemum will more strongly influence plant growth in the second cycle than the effects of initial inoculation with plant-conditioned soil; and 3) inoculation with plant-conditioned soils that have negative effects on chrysanthemum will increase the negative effects of introduced soil-borne diseases in the second growth cycle, while plant-conditioned soil inocula with positive effects on chrysanthemum growth will suppress the effects of soil-borne diseases in the second growth cycle. 4) Plant growth in the second cycle will be more strongly influenced by soil-borne diseases added in the first than in the second growth cycle.

Materials and methods

Plant material

The focal plant in our study was *Dendranthema X grandiflora* (Ramat.) Kitam. cv. Grand Pink (Chrysanthemum, syn. *Chrysanthemum X morifolium* (Ramat.) Hemsl., Asteraceae). Chrysanthemum cuttings were provided by the breeding company FIDES by Dümmen Orange (De Lier, The Netherlands).

Pathogen propagations

The soil-borne oomycete pathogen *Pythium ultimum* (Pythiaceae) was obtained from Wageningen UR Greenhouse Horticulture (Wageningen UR, Greenhouse Horticulture, Bleiswijk, The Netherlands). *Pythium ultimum* was isolated from chrysanthemum plants, and cultured on liquid V8 medium (200 ml of organic tomato suspension without added salt, 2 g CaCO3, and 800 ml water) at room temperature for 2 weeks. The *P. ultimum* culture was then blended in a mixer and filtered to obtain a solution with only oospores based on a modified protocol of van der Gaag and Wever (2005). The oospores concentration was determined by counting the number of oospores in 1 ml liquid suspension under the microscope using a Fuchs-Rosenthal chamber.

Meloidogyne incognita J2 nematodes were obtained from HZPC Holland B.V., The Netherlands. The purity of the culture was assessed with species-specific markers by AgroXpertus (Wageningen, The Netherlands). The culture contained 99% *M. incognita* and 1% *M. hapla*.

Diseased soil was obtained from a commercial chrysanthemum greenhouse in Made, The Netherlands. The soil from this commercial greenhouse contained high densities of *Meloidogyne incognita*.

Experimental set-up

The experiment consisted of three phases. In the conditioning phase, eight plant species were used to condition soil individually: *Anthoxanthum odoratum*, Poaceae (AO), *Bromus hordeaceus*, Poaceae (BH), *Festuca filiformis*, Poaceae (FF), *Lolium perenne*, Poaceae (LP), *Holcus lanatus*, Poaceae (HL), *Rumex acetosella*, Polygonaceae (RA), *Galium verum*, Rubiaceae (GV) and *Hypochaeris radicata*, Asteraceae (HR). For the next two growth cycles, chrysanthemum plants were grown repeatedly either in sterile soil inoculated with plant-conditioned soil or in sterile soil (un-inoculated), and either with exposure to different disease treatments in each cycle or not exposed (control). Disease treatments added in cycle 1 (*Pythium* or *Meloidogyne* or control) were termed as "disease 1", disease treatments added in cycle 2 (*Pythium* or diseased soil inoculum or control) were termed as "disease 2". The soils that were used at the start of cycle 1 as inoculum and conditioned by plant monocultures were termed "plant-conditioned inocula".

In cycle 2, there were nine combined disease treatments, as a result of the full factorial combination of treatments in the first and the second cycle. Treatments were abbreviated with codes consisting of two letters, the first one represents the disease treatment applied during the first cycle and the second one represents the treatment imposed during the second cycle: control – control (C-C), control – *Pythium* (C-P), control – diseased soil inoculum (C-D), *Pythium* – control (P-C), *Pythium*- *Pythium* (P-P), *Pythium* – diseased soil inoculum (P-D), *Meloidogyne* – control (M-C), *Meloidogyne* – *Pythium* (M-P), *Meloidogyne* – diseased soil inoculum (M-D). In addition, in cycle 2, the three disease treatments (control, *Pythium*, diseased soil) were also imposed to new sterilized soil making a total of 12 treatments in cycle 2. A schematic drawing of the experiment is presented in Fig. 4.1.

Phase I: Conditioning phase

For the conditioning phase, soil was collected (5–20 cm deep) in June 2015 from a semi-natural grassland on former arable land (Mossel, Ede, The Netherlands). The field had been used for agricultural purpose until 1996. The sandy-loam soil was homogenized and sieved (1 cm mesh size) to remove coarse fragments and all macro-arthropods. Pots (13 × 13 × 13 cm) were filled with a homogenized mixture of field soil and sterilized field soil in a 1:1 ratio. Soil sterilization was done by gamma irradiation (> 25 K Gray gamma irradiation, Isotron, Ede, The Netherlands). The sterilized soil was added to minimize potential differences in soil nutrient heterogeneity and to provide a niche for the soil microbes to grow and hence increase the potential for plant-species-specific effects on the soil community. Pots were filled with 1.6 Kg of soil (based on dry weight).

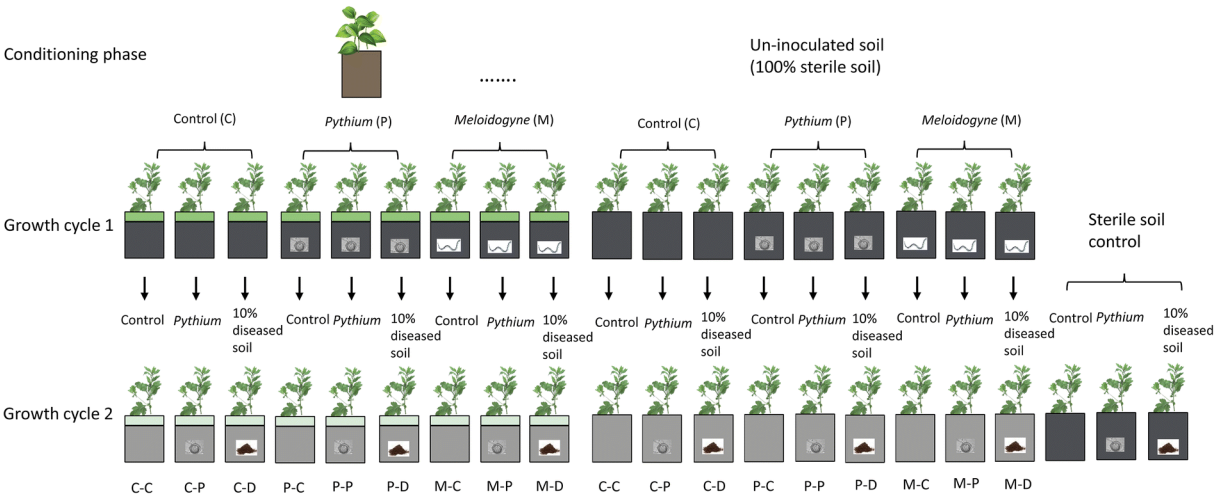

Fig. 4.1 Experimental design. Eight plant species were grown in monocultures for 10 weeks, for clarity, only one of the eight species is depicted. In growth cycle 1, chrysanthemum was planted in 90% sterile soil inoculated with 10% plant conditioned soil. A set of replicates with un-inoculated soil (100% sterile soil) was also included. Each soil treatment was further divided in three disease treatments: *Pythium*, *Meloidogyne* or control. In growth cycle 2, all soils from the previous cycle were used for a second round of chrysanthemum growth. Each treatment combination from cycle 1 was divided into pots receiving *Pythium*, 10% diseased soil inoculum, or control pots. In cycle 2, a new set of replicates with 100% sterile soil was included and these were also imposed to disease treatments.

Seeds of all wild plant species were obtained from a wild plant seed supplier (Cruydt-Hoeck, Assen, The Netherlands). Seeds were surface sterilized in 3% sodium hypochlorite solution for 1 min, rinsed and germinated on sterile glass beads in a climate chamber at 20 °C (16 h/8 h, light/dark). Five one-week-old seedlings were transplanted in monocultures in each pot, and there were ten replicate pots for each species. In total, the conditioning phase comprised of 80 pots (monocultures of 8 plant species × 10 replicates). Seedlings that died during the first week of the experiment were replaced. As a few seedlings died later, the number of seedlings in each pot was reduced to four so that the density was the same in all pots. All pots were placed randomly in a climate controlled greenhouse with 70% RH, 16 h at 21 °C (day) and 8 h at 16 °C (night). Natural daylight was supplemented by 400 W metal halide lamps (225 µmol $s^{-1}m^{-2}$ photosynthetically active radiation, one lamp per 1.5 m^2). The pots were watered regularly. Ten weeks after transplanting, the plants were carefully removed from each pot and the largest roots were removed from the soil as they may act as a source for re-growing plants. Finer roots were left in the soil as the rhizosphere around these roots may include a major part of the microbial rhizosphere community. The soil from each pot was homogenized and stored separately in plastic bags at 4 °C until used in the test phase so that there were 10 replicate soils for each plant species.

Phase II: Growth cycle 1

For growth cycle 1, 1 L pots (11 × 11 × 12 cm; length × wide × height) were filled with a homogenized mixture of 10% soil inoculum (plant species-specific conditioned soil) and 90% sterilized soil (see above). Pots filled with 100% sterilized soil served as control (un-inoculated soil). Two chrysanthemum cuttings (without roots) were planted in each pot as preliminary work showed that not all cuttings establish properly with this method. Prior to planting, the soil in each pot was watered and 100 ml half-strength Hoagland nutrient solution was added. The pots were placed on trolleys, each trolley had 48 pots and was tightly covered with a thin transparent plastic film for 10 days to create a closed environment with high humidity that favors rooting. After 10 days, one of the chrysanthemum cuttings was removed from each pot. Seven days after the transparent plastic film had been removed, 2 ml of the oospore suspension (ca. 60,000 oospores of *P. ultimum*) was added onto the soil next to the stem of each plant allocated to the *Pythium* treatment. A 1.5 cm deep hole was made in the soil a near the stem of each plant allocated to the nematode treatment, and 5 ml suspension containing *M. incognita* (ca. 5900 Juveniles stage 2) was added. Plants were fertilized following common practices used by chrysanthemum growers: half-strength Hoagland nutrient solution for the first two weeks, single-strength Hoagland solution during the following two weeks. The strength was increased to 1.6 mS/cm EC (electrical conductivity) for the last two weeks. The density of pots on each trolley was reduced two weeks after the start of the second phase to 32 pots per trolley so that there was 10 cm space between each pot. There were three replicate pots for each soil from the conditioning phase. Hence, cycle 1 comprised of 810 pots (8 plant species-specific soil inocula × 3 disease treatments × 10 soil replicates × 3 pot replicates + non-inoculated soil × 3 disease treatments × 10 soil replicates × 3 pot replicates). All pots were randomly arranged in a greenhouse compartment and kept under the same conditions as described for the conditioning phase.

Six weeks after rooting, all plants were harvested. Plants were clipped at soil level and roots were removed from the soil and the soil was returned to each pot for the next grow cycle. Roots were washed over a sieve (2 mm mesh). For each plant, leaf yellowness was recorded as a plant health indicator, because in chrysanthemum leaf yellowness is symptomatic for diseases such as those caused by soil pathogens like *Verticillium*, *Fusarium* (Reddy 2015). All leaves were counted on each plant and the number of leaves that showed yellowness (partly, or completely) was recorded. Leaf yellowness was calculated as the proportion of leaves that showed yellowness. Root color was also recorded at a scale of 0 to 3, where 3 indicates a dark and diseased root system, and 0 indicates a white/light colored and healthy root system (photos of root systems illustrative for the root darkness categories are presented in Fig.S4.1). Root galls caused by root knot nematodes were scored with a scale of 0 to 10 (Dhandaydham et al. 2008). 0 = no visual galling, 1 = less than 5% small galls, 2 = 10% small galls, 3 = 15% small galls, 4 = 20% large galls, 5 = 30% large galls, 6 = 40% big galls, 7 = 50% big galls, 8 = 60% big galls, 9 = 70% big galls, 10 = more than 75% big galls. In cycle 1, nematode scores were only recorded for

chrysanthemum roots from plants exposed to the *Meloidogyne* treatment, but in cycle 2, this was done for all plants from all treatments. Shoot and root biomass was then oven-dried (60 °C for 3 days) and weighed.

Phase III: Growth cycle 2

The experimental procedure was as described for cycle 1. In this phase, we used as disease treatments *P. ultimum* addition (6 × 10^4 oospores), 10% "diseased" soil, *i.e.* soil collected from a commercial chrysanthemum greenhouse with severe soil disease problems, and control. Two new unrooted chrysanthemum cuttings were planted into each pot from growth cycle 1 as described above. A new set of 30 pots filled with 100% sterilized soil was included during growth cycle 2, either with 10% diseased soil, or 60,000 oospores of *P. ultimum*, or control. This phase comprised of the same 810 pots plus 10 replicates for each of the 3 soil disease treatments using new 100% sterile soil, resulting in a total of 840 pots. Six weeks after rooting, plants were harvested as described above.

Statistical analysis

After growth cycle 1, the overall effects of the plant-conditioned soil inocula and disease treatments on chrysanthemum shoot biomass and root biomass were determined using a linear mixed model. In the model, "soil identity" and "disease treatment 1" were used as fixed factors, and soil replicate was used as random factor. *Post-hoc* Tukey tests were used for pairwise comparisons between plant-conditioned soil inocula. The same analysis was also performed including un-inoculated soil. A *post-hoc* Dunnet test was used to compare chrysanthemum biomass for each of the plant-conditioned soil inocula with that of un-inoculated soil. As chrysanthemum leaf yellowness is proportional data, a generalized linear mixed model with a binomial distribution and logit link function was used. Data on root darkness are categorical, therefore a generalized linear mixed model with Poisson distribution was used. Nematode scores were only recorded for plants in the *Meloidogyne* treatment, therefore, a generalized linear model was used to determine the overall effects of the soil inoculation treatments on nematode infection.

For data from growth cycle 2, the effects of the plant-conditioned soil inocula and disease treatments on chrysanthemum shoot biomass and root biomass were determined using a linear mixed model. In the model, "soil identity", "disease treatment 1" and "disease treatment 2" were used as fixed factors, and soil replicate was used as random factor. A *post-hoc* Tukey test was used for pairwise comparisons among the disease treatments of both cycles. For root darkness and root nematode scores, a generalized linear mixed model with Poisson distribution was used to examine the overall soil effects (analyzed

separately with and without un-inoculated soil) and effects of disease treatments in cycle 1 and disease treatments in cycle 2.

We then compared the effects of the three disease treatments imposed during cycle 2 on chrysanthemum performance in (i) soils inoculated in cycle 1, (ii) soils that were not inoculated in cycle 1, and (iii) new sterile soil. We used the average for the inoculated soils as the differences between the effects of plant-conditioned inocula on chrysanthemum in cycle 2 were less variable compared with their effects in cycle 1 (see results). For inoculated and un-inoculated soils from cycle 1, there were 9 combinations of disease treatments (3 for cycle 1 × 3 for cycle 2) while for new sterile soil there were only 3 treatments (3 for cycle 2). The disease treatments added to inoculated and un-inoculated soil in cycle 1 were therefore grouped based on the three disease treatments from cycle 2. C-C, P-C and M-C of inoculated and un-inoculated soil were compared to the C in new sterile soil; C-P, P-P and M-P were compared to P, and C-D, P-D and M-D to D. One-way ANOVA was then used to compare these seven groups and a *post-hoc* Tukey test was used for pairwise comparisons. These analyses were done for chrysanthemum shoot biomass, root biomass and leaf yellowness. As leaf yellowness were recorded as a proportion, a generalized linear model was used instead.

Additionally, chrysanthemum shoot biomass, root biomass and yellowness were grouped based on the soil treatment (soils inoculated in cycle 1 and soils that were not inoculated in cycle 1), and a linear mixed model was then used to examine the impact of the disease treatments. In this model, the disease treatments were used as fixed factor, and soil replicate was used as random factor. For sterile soil, one-way ANOVA was used to examine the impact of disease treatments. Within each disease treatment, chrysanthemum shoot and root biomass of plants growing in different inoculated soil was compared using one-way ANOVA. The same analysis was done for chrysanthemum leaf yellowness, but either with a generalized linear model or a generalized linear mixed model with binomial distribution and logit link function. To determine the effects of the disease treatments within each soil, a generalized linear model was used to compare chrysanthemum root darkness and root nematode score between different disease treatments. We also used linear regression to determine the relationship between root biomass of conditioning plant species and root biomass of chrysanthemum grown later in the conditioned soils. This analysis was done for root biomass of chrysanthemum in both cycle 1 and cycle 2. All analyses were performed in R (version 3.0.1, R Development Core Team 2017).

Results

After growth cycle 1, chrysanthemum shoot biomass, root biomass and leaf yellowness significantly differed between plant-conditioned inocula, while "disease treatment 1" only significantly influenced leaf yellowness. Root darkness and the root nematode scores were not significantly affected by any of the treatments (Table 4.1, Table S4.1, Fig. S4.2). When un-inoculated soil was included in the analysis, the same effects were significant (Table S4.1). Plants grown with AO inoculum sustained higher shoot and root biomass than plants with HR inoculum, and AO, BH, GV, and LP inocula sustained significantly higher shoot biomass than un-inoculated soil (Fig. 4.2a, b). Root biomass of plants grown with plant-conditioned inocula did not differ from root biomass in un-inoculated soil (Fig. 4.2b). In the control, without addition of diseases, the proportion of yellow leaves, was significantly lower with AO, BH, or HL inocula than with un-inoculated soil, and did not differ between plant-conditioned soil inocula. Plants exposed to *Meloidogyne* and growing with AO, BH, GV inocula had a significantly lower proportion of yellow leaves, than plants grown with *Meloidogyne* and RA inoculum. In presence of *Meloidogyne*, none of the plant-conditioned inocula were significantly different from un-inoculated soil. When exposed to *Pythium*, plants with AO and FF inocula had a significantly lower proportion of yellow leaves than plants grown in un-inoculated soil, and there were no significant differences between plant-conditioned inocula (Fig. 4.2c).

After growth cycle 2, there were no significant interactions between any factors on shoot biomass or root biomass. Root biomass and leaf yellowness differed significantly between plant-conditioned inocula (Table 4.2; Fig. 4.3). Shoot biomass and leaf yellowness were significantly influenced by "disease treatment 1". Shoot biomass and leaf yellowness of plants grown in soil where *Meloidogyne* were added in cycle 1 was higher than in plants grown in soil from the control treatment from cycle 1. Plants growing in soil inoculated with diseased soil in cycle 2 had higher root biomass than those growing in soil from the control and the *Pythium* treatment. Plants exposed to *Pythium* in cycle 2 in general had significantly lower leaf yellowness than plants from the control treatment. Leaf yellowness varied between disease and soil treatments resulting in significant interactions between "disease treatment 1" and "soil", "disease treatment 1" and "disease treatment 2", and between "disease treatment 1", "disease treatment 2" and "soil" (Table 4.2, Fig. 4.3c). In the *Pythium* treatment in cycle 1 yellowness was higher in inoculated soil that had been conditioned by LP and RA, and lower in soil conditioned by HL. For AO, GV, and HR and un-inoculated soil, yellowness was higher in the control treatment in cycle 1 than in other disease treatments, while in cycle 2 this was true for the control treatment for soil conditioned by BH, HL and LP. Yellowness in the diseased soil treatment in cycle 2 was higher when plants were grown in soil conditioned by FF and RA. Exposure to *Pythium* in both cycles led to higher yellowness in RA soil, while exposure first to *Meloidogyne* and then *Pythium* led to higher yellowness

Table 4.1 Effects of plant-conditioned soil inocula and disease treatment 1 on chrysanthemum shoot biomass, root biomass and leaf yellowness at the end of cycle 1. "Species" indicates the identity of the conditioning plant species. "Treatment 1" indicates the disease treatments in cycle 1. Presented are F-values obtained from a mixed linear model (generalized mixed linear model for leaf yellowness). *** indicates significant difference at $P < 0.001$.

	Df	Shoot biomass	Root biomass	Yellowness
Species	7,72	7.751***	4.678***	5.708***
Treatment1	2,624	1.101	0.263	8.240***
Treatment1*Species	14,624	1.472	1.113	5.694***

in AO soil and in un-inoculated soil (Table 4.2; Fig. 4.3c). Plants grown in diseased soil had healthier roots (lower root darkness scores) than plants from the *Pythium* and control treatment. However, the root nematode score was significantly higher for plants inoculated with diseased soil in cycle 2 than for other plants (Table S4.2; Fig.S4.3). When un-inoculated soil was included in the same analysis, the results were similar, except that for this analysis, "disease treatment 1" effects were significant for chrysanthemum root biomass (Table S4.3).

We subsequently analyzed the ten soil categories separately (the eight plant-conditioned soil inocula, the un-inoculated soil from cycle 1, and new sterile soil). Shoot biomass of plants grown in LP and un-inoculated soil from cycle 1 and in new sterile soil differed significantly between disease treatments (Fig. 4.3a). Root biomass of plants in all soil categories, except for HL inoculum, differed significantly between disease treatments (Fig. 4.3b). Overall, in soils from cycle 1 (8 plant-conditioned inocula and un-inoculated soil), chrysanthemum grown in pots where diseased soil was added had more biomass than chrysanthemum exposed to the other disease treatments. At the end of cycle 2, for plants grown in un-inoculated soil from cycle 1, biomass in the double control treatment (C-C) was lower than in the other treatments. For chrysanthemum grown in sterile soil in cycle 2, addition of diseased soil led to a reduction in biomass, and *Pythium* addition did not affect biomass. In cycle 2, root darkness did not differ between disease treatments within each soil category (Fig.S4.3a). However, nematodes scores in plants grown in pots where diseased soil was added were much higher than in plants from other treatments (Fig.S4.3b). Figures and statistical analyses presenting the ten soil categories for each of the nine disease treatment combinations are shown in the appendix (Fig.S4.4, Table S4.4, S4.5).

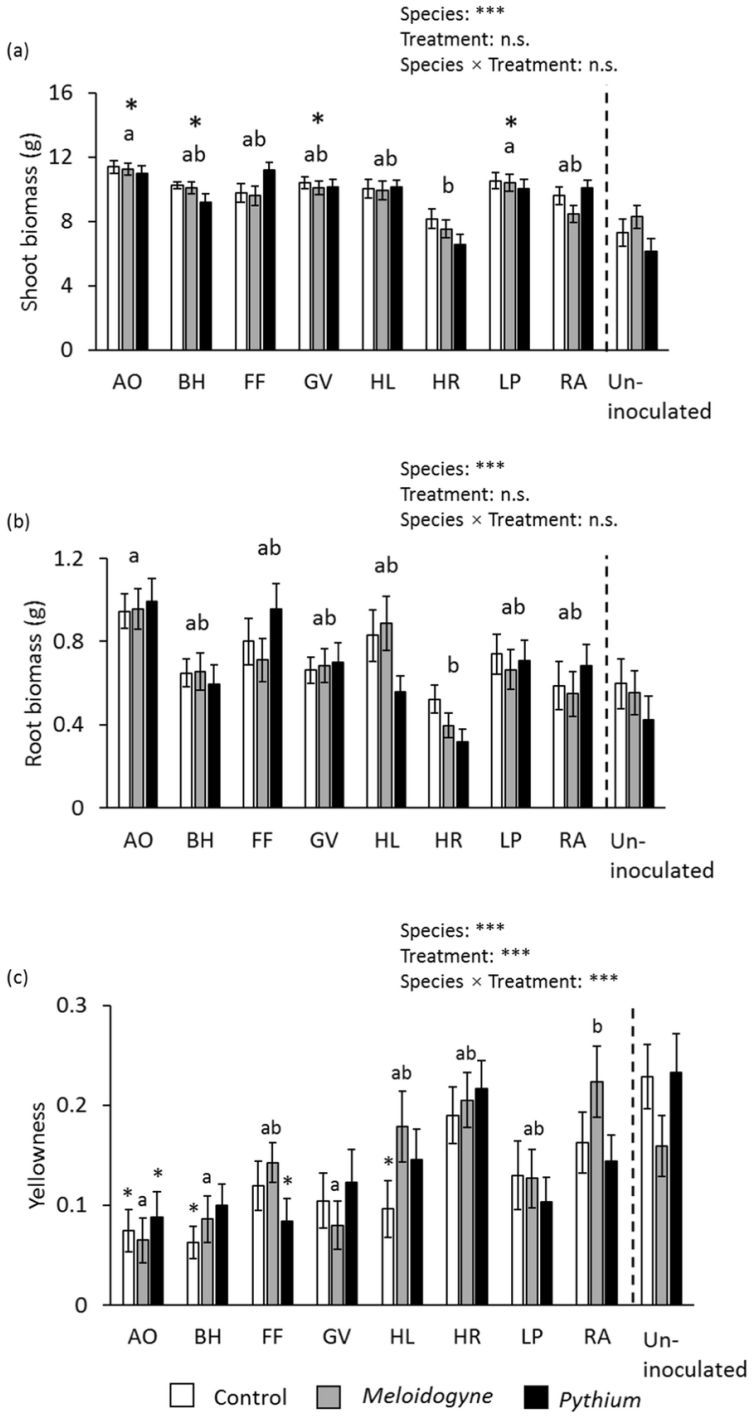

Fig. 4.2 Chrysanthemum shoot biomass (a), root biomass (b) and leaf yellowness (c) in plant-conditioned soil inocula with different disease treatments after growth cycle 1. White bars indicate chrysanthemum performance in the control treatment, grey bars the *Meloidogyne* treatment, and black bars the *Pythium* treatment. Statistics presented in the upper part of each panel are the overall effects of the plant-conditioned inocula and disease treatment 1 from a mixed linear model (generalized mixed linear model for leaf yellowness). *** indicates significant difference at $P < 0.001$. "n.s." indicates no significant difference detected. Different letters above each set of bars indicate significant difference between plant-conditioned soil inocula ($P < 0.05$). * indicates significant difference of a plant-conditioned inoculum and the un-inoculated soil ($P < 0.05$).

Table 4.2 Overall effects of plant-conditioned soil inocula, disease treatment 1, and disease treatment 2 on chrysanthemum shoot biomass, root biomass and leaf yellowness at the end of cycle 2. "Species" indicates the identity of plant species that conditioned the soil used for the inoculum. "Treatment 1" indicates the disease treatments imposed during cycle 1, "Treatment 2" indicates the disease treatments added in cycle 2. Presented are F-values obtained from a linear mixed model (generalized linear mixed model for leaf yellowness). For pairwise comparisons between each category of disease treatments T-values are presented (Z-value for leaf yellowness). *,**,*** indicates significant differences at $P < 0.05$, 0.01 or 0.001, respectively.

	Df	Shoot biomass	Root biomass	Yellowness
Species	7,72	1.937	2.568*	2.147*
Treatment1	2,576	3.081*	2.425	9.190**
Control vs *Meloidogyne*		-2.292*	-2.187	2.638*
Control vs *Pythium*		-0.320	-0.868	0.097
Meloidogyne vs *Pythium*		1.972	1.318	0.055
Treatment2	2,576	0.869	52.441***	71.139***
Control vs Diseased soil		-0.101	-9.463***	0.248
Control vs *Pythium*		-1.189	-1.339	2.253*
Diseased soil vs *Pythium*		-1.088	8.123***	-0.220
Treatment1*Species	14,576	1.155	0.868	4.401*
Treatment2*Species	14,576	0.833	0.496	2.335
Treatment1*treatment2	4,576	0.676	0.671	8.944*
Treatment1*Treatment2*Species	28,576	1.053	0.839	3.725*

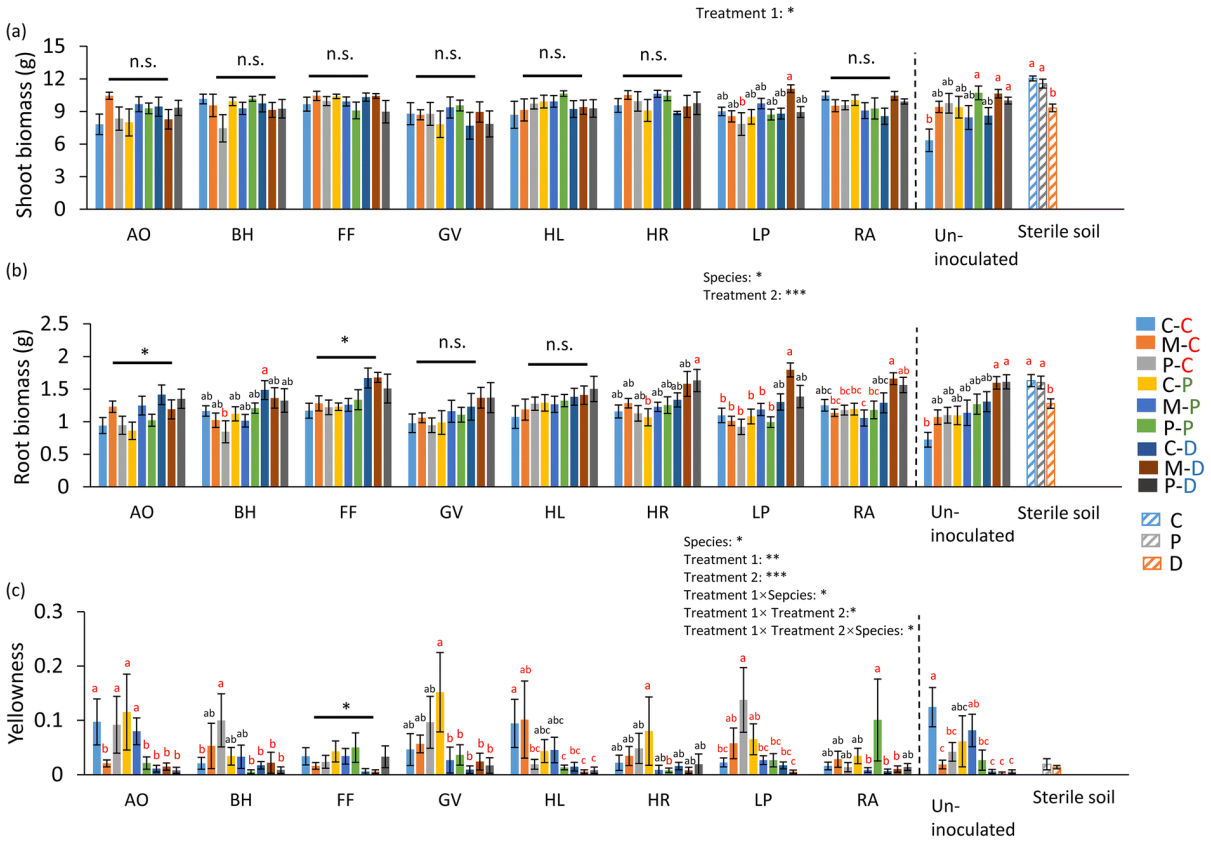

Fig. 4.3 Chrysanthemum shoot biomass (a), root biomass (b) and leaf yellowness (c) in plant-conditioned soil inocula with different disease treatments after growth cycle 2. Colors of the bars indicate the different inoculation and disease treatment combinations. "*" indicates that for this specific soil inoculum there is an overall significant effect of disease treatments, however individual treatments do not significantly differ. Statistics presented in the upper part of each figure are the significant effects, more details are presented in Table 4.2. For inocula with different letters above bars there is an overall significant effect of disease treatments and significant differences among the treatments are highlighted with red letters. "n.s." indicates no significant difference detected. Statistics of each soil category are presented in Table S4.4.

In cycle 2, on average, chrysanthemum shoot biomass and root biomass was higher in pots that were inoculated in cycle 1 with plant-conditioned soil than in pots that were not inoculated in cycle 1 (Fig. 4.4a, d). However, in soil where chrysanthemum had been grown before, both in pots inoculated with plant-conditioned soil and in un-inoculated soil, shoot and root biomass was lower in cycle 2 than in sterile soil for the control (Fig.4.4a, d) and *Pythium* treatment (Fig. 4.4b, e) in cycle 2. For plants grown in pots where diseased soil was added in cycle 2, there were no significant differences for shoot and root biomass between the three types of soils (cycle 1 inoculated, cycle 1 un-inoculated, cycle 2 sterile; Fig. 4.3c, f). Leaf yellowness did not significantly differ among plants grown in the different types of soils (Fig. 4.4g–i). Moreover, there was no significant relationship between root biomass of the conditioning plant species and root biomass of chrysanthemum in both cycle 1 and cycle 2 (Fig.S4.5).

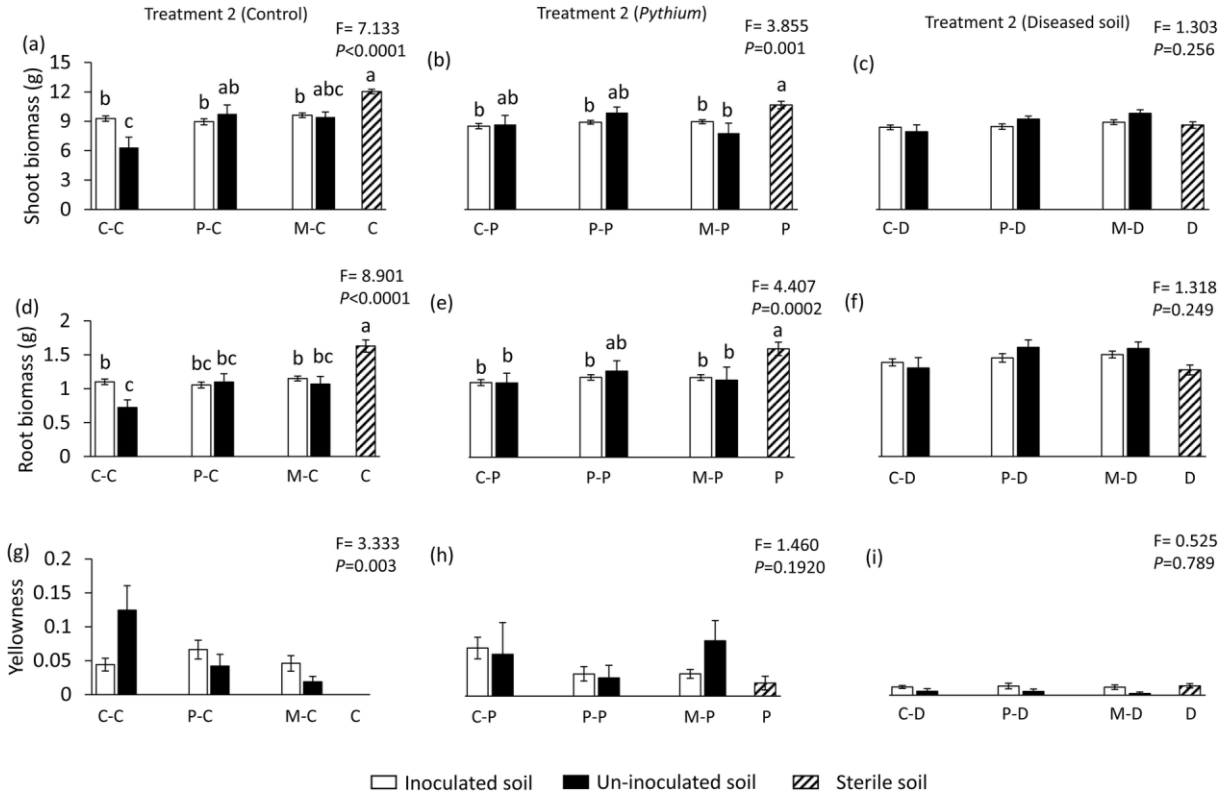

Fig. 4.4 Average values for inoculated soil (average of the 8 inocula), un-inoculated soil (100% sterile in cycle 1) and new sterile soil (100% sterile soil in cycle 2) for chrysanthemum shoot biomass (a–c), root biomass (d–f) and leaf yellowness (g–i) at the end of cycle 2. White bars indicate average chrysanthemum performance in soil inoculated with plant-conditioned inocula, black bars indicate performance in un-inoculated soil from cycle 1, striped bars indicate performance in sterile soil from cycle 2. For each plant parameter, the figure is grouped by disease treatments imposed in cycle 2 and split into three panels. In each panel, a one-way ANOVA was used to test the overall differences between all the bars, and a post hoc Tukey test was used to do pairwise comparisons between bars. Bars with identical letters are not significantly different. F-value and *P* value of one-way ANOVAs are presented in the upper part of each panel.

Discussion

Our results show that the effects of soil inoculation at the start of cycle 1 were still detectable after the second growth cycle. After two growth cycles, chrysanthemum generally grew better in new soil (sterile soil) than in soils in which chrysanthemum had been grown before (*i.e.* in plant-conditioned soil and in un-inoculated soil), indicating that this species exhibits a negative conspecific plant-soil feedback. Importantly, inoculating sterilized soil with plant-conditioned soil reduced this negative conspecific feedback effect, as plant growth in cycle 2 in plant-conditioned soil was better than in un-inoculated soil. Moreover, in cycle 2, the effects of plant-conditioned inocula on plant growth were less variable than in cycle 1, suggesting that growth of chrysanthemum in all soils for one cycle caused all plant-conditioned

soils to develop in a similar way. Remarkably, inoculation with soil from a commercial greenhouse that contained diseases had a strong negative effect on plant growth when this was done in sterile soil, but when this "diseased soil" was added to soils in which chrysanthemum had been grown before, it increased plant growth.

In accordance with the first hypothesis, the effects of adding inocula that consisted of soil in which another plant had been grown (plant-conditioned inocula) were still present in the second growth cycle. This is in line with other studies that show that plant-mediated changes in the soil can affect the performance of other succeeding plants in both agricultural and natural ecosystems (de la Peña et al. 2016; Jangid et al. 2011; Wubs and Bezemer 2018). In general, the mechanisms for these effects are difficult to disentangle because they can be due to changes in soil abiotic and biotic conditions (de la Peña et al. 2016). In this study, we found that addition of 10% of a soil inoculum to sterile soil influenced chrysanthemum growth during two cycles. The small amount of soil inoculum added, and the high fertilization rates in all treatments, make it unlikely that soil nutrient availability played a role, and hints at a pivotal role of the soil microbial community (Brinkman et al. 2010). Extended impacts of the soil microbial community on plant health, have also been reported in studies on soil disease suppression, where suppressiveness of the soil against pathogens could be maintained for several growth cycles (Lapsansky et al. 2016; Janvier et al. 2007; Mendes et al. 2011). In the current study, we only tested the effects of soil inoculation for two growth cycles. How long these effects of the soil inocula persist, and whether and how they influence chrysanthemum performance in subsequent growth cycles is unknown and this is an important question to be addressed in future studies.

In accordance with the second hypothesis, chrysanthemum, which was the most recently growing species in the soil, had a stronger influence on biomass in cycle 2 than the plant species that conditioned the soil that was used for inoculation at the start of cycle 1. Additionally, we also observed that chrysanthemum grew better in new sterile soil than in soil in which it had been grown before. This indicates that chrysanthemum exhibits a negative feedback, due to the build-up of pathogens in the rhizosphere or due to the release of plant metabolites in the soil that inhibit plant growth (*i.e.* autotoxic effects, Zhou et al. 2009). Inoculation of the sterilized soil with soil that was conditioned by another plant species reduced this negative plant-soil feedback effect. Such negative conspecific feedbacks are a commonly observed problem in continuous cropping systems (Song et al. 2013; Zhou et al. 2009; Wang et al. 2017b). However, we now show that adding an inoculum consisting of soil conditioned by other plant species to the sterilized soil before the first growth cycle starts can reduce this negative effect. Previous studies have reported that certain soil microbiomes have disease-suppressive properties in soils (Schlatter et al. 2017; Mendes et al. 2011; Ridout and Newcombe 2016; Berendsen et al. 2012). Our

study further shows that over successive growth cycles, microbiomes of unrelated plant species can be used to reduce negative conspecific plant-soil feedbacks in horticultural crops. This could be achieved by changing the soil via growing another crop intermediately, or by soil inoculation, as in our study. Our study indicates that addition of soil conditioned by other plant species to sterilized or steamed soil could potentially increase crop yield of later harvests, as well as increase the number of crops that can be harvested sequentially before the soil has to be steamed again. Steaming of soil exhibits a significant environmental footprint (Ispahani et al. 2008) and an additional harvest before the grower has to steam the soil again would increase the sustainability of the crop, which is an important aim in the chrysanthemum sector (Kos et al. 2014).

To answer the third hypothesis, the plant-conditioned soil inocula should be characterized as positive and negative. However, compared to control soil, in the first cycle there were only positive to neutral effects of inoculation with plant-conditioned soil, and the difference among the effects of plant-conditioned soil inocula was less variable in the second than in the first cycle. For example, in cycle 1, addition of soil conditioned by the plant species *Hypochaeris radicata* (HR) had the most negative influence on chrysanthemum, while inoculation with from soil conditioned by *Anthoxanthum odoratum* (AO) resulted in the greatest biomass. In the second cycle, this difference between AO and HR disappeared. Other studies on the temporal dynamics of plant-soil feedback effects have argued that the changes in plant-soil feedbacks over time will depend on the target plant species in the feedback phase (Kardol et al. 2006, 2013; Hawkes et al. 2013). In this study, the effects of all inocula converged over time. This is probably because of the overriding effects of chrysanthemum on the soil. We did not examine the microbial community of the different inocula and how much of these differences remained present after chrysanthemum had grown in the inoculated soils. Future studies should test how the composition of the soil microbial communities changes due to inoculation with plant-conditioned soil and how this is subsequently influenced by the growth of chrysanthemum in these soils.

In our study, addition of *Pythium* and *Meloidogyne* did not have consistent negative effects on plant growth and health. After cycle 2, leaf yellowness differed significantly between soil treatments. However, this pattern was not consistent among the disease treatments imposed in cycle 1 or 2. Moreover, for some soils and in both cycles, the yellowness of plants in the control treatments was significantly higher than in disease treatments, suggesting that the disease treatments may even reduce yellowness. Although leaf yellowness is a health indicator in chrysanthemum (Reddy 2015), it is not caused specifically by *Pythium* or *Meloidogyne*, and other pathogens that may have been present in the soil inocula or in the diseased soil could also cause leaf yellowing. On the basis of these results, conclusions regarding the fourth hypothesis cannot be made. The spore and juvenile density of *Pythium*

and *Meloidogyne* used in this study are sufficient to cause symptoms in plant growth (van der Wurff et al. 2010) but we speculate that the virulence of the disease inocula was limited. This is supported by the low nematode and darkness scores in the roots and the lack of differences among disease treatments in these scores. It is also possible that the *Pythium* and *Meloidogyne* inoculations were ineffective *e.g.* because these pathogens did not establish successfully in the soil or were outcompeted by microbes present in the soil. An interesting finding of our study is that the addition of diseased soil in cycle 2 significantly increased performance of chrysanthemum in soils where chrysanthemum had been grown before, but that it had a negative effect on biomass when added to new sterile soil. Plant performance in the soils in which chrysanthemum had been grown before was worse than in new sterile soil. Hence, a negative treatment (*i.e.* diseased soil) was imposed to soils where plant performance was reduced already. This phenomenon that addition of a negative treatment to a soil which already has a negative effect is similar to a concept of soil immunity (Raaijmakers and Mazzola 2016). However, soil immunity typically appears after an outbreak of a soil-borne disease, and it takes a long period of mono-cropping, up to decades, to achieve soil immunity (Hamid et al. 2017; Raaijmakers and Mazzola 2016). Whether, the soils from cycle one in our study have become immune to soil diseases is unknown, and further work is required to disentangle these plant-soil feedback and soil disease interactions.

In conclusion, this study provides a possible application of the plant-soil feedback concept in horticulture, and demonstrates that inoculation of sterile soil with live soil conditioned by other plant species can reduce but not completely remove the negative conspecific plant-soil feedback of chrysanthemum. We did not observe strong effects of addition of soil diseases. Plant growth was best in sterile soil but, importantly, this effect disappeared when plants were grown in pots where soil was added from a commercial greenhouse with soil disease problems. Future studies should unravel the role of the composition of the microbiome, and mechanisms behind the soil inoculation effects, as well as test the effects of soil inoculation with whole microbiomes in a real horticultural greenhouse scenarios.

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Supplementary material

Table S4.1 The effects of soil inoculation (plant-conditioned soil inocula and un-inoculated soil) and disease treatment 1 on chrysanthemum shoot biomass, root biomass, leaf yellowness, root darkness and nematode infestation score. Presented are F values from a linear mixed model (generalized mixed linear model for leaf yellowness and root darkness, generalized linear model for nematode score). Nematode score were only measured for plants in the *Meloidogyne* treatment. **,*** indicate significant difference at $P<0.01$ or 0.001, respectively.

	Df	Shoot biomass	Root biomass	Yellowness	Root darkness	Nematode score
Soil	8,72	7.870***	4.216***	5.234***	1.209	0.150
Treatment1	2,711	0.867	0.522	3.185**	0.670	N.A
Treatment1*Soil	16,711	1.613	0.995	6.597***	0.382	N.A

Table S4.2 Overall effects of plant-conditioned soil inocula, disease treatment 1 and disease treatment 2 on chrysanthemum root darkness and nematode scores. "Species" indicates the identity of conditioning plant species. Presented are F-values following a generalized mixed linear model. For pairwise comparisons between each category of disease treatments Z-values are presented. *,**,*** indicates significant difference at $P<0.05$, 0.01 or 0.001, respectively.

	Df	Root darkness	Nematode score
Species	7,72	0.082	1.072
Treatment1	2,576	0.237	1.264
Control vs *Meloidogyne*		-0.251	0
Control vs *Pythium*		0.416	0
Meloidogyne vs *Pythium*		0.668	0
Treatment2	2,576	5.281*	71.906***
Control vs Disease soil		3.316**	-0.005
Control vs *Pythium*		0.791	0
Disease soil vs *Pythium*		-2.530*	0
Treatment1*Species	14,576	0.287	0.644
Treatment2*Species	14,576	0.234	0.203
Treatment1*treatment2	4,576	0.567	0
Treatment1*Treatment2*Species	28,576	0.276	0

Table S4.3 Overall effects of soil inoculation (plant-conditioned soil inocula and un-inoculated soil), disease treatment 1, and disease treatment 2 on chrysanthemum shoot biomass, root biomass, leaf yellowness, root darkness and nematode scores at the end of cycle 2. Presented are F-values from a mixed linear model (generalized mixed linear model for leaf yellowness, root darkness and nematode scores), for pairwise comparisons between each category of disease treatments, T-values are presented (Z-value for leaf yellowness, root darkness and nematode scores). *,**,*** indicates significant difference at $P<0.05$, 0.01 or 0.001, respectively.

	Df	Shoot biomass	Root biomass	Yellowness	Root darkness	Nematode score
Soil	8,72	1.697	2.251*	1.962*	0.101	0.940
Treatment1	2,657	4.258*	3.888*	9.962**	0.406	1.064
Control vs *Meloidogyne*		-2.918*	-2.761*	3.029**	-0.547	0
Control vs *Pythium*		-1.461	-1.722	0.065	0.346	0
Meloidogyne vs *Pythium*		1.458	1.039	0.032	0.893	0
Treatment2	2,657	1.415	63.403***	84.331***	5.046*	82.515***
Control vs Disease soil		-0.804	-10.563***	0.179	3.217**	-0.005
Control vs *Pythium*		-1.682	-1.903	2.185	0.804	0
Disease soil vs *Pythium*		-0.878	8.660***	-0.160	-2.418*	0
Treatment1*Soil	16,657	1.615	1.074	4.621*	0.267	0.671
Treatment2*Soil	16,657	0.957	0.643	2.497	0.242	0.251
Treatment1*treatment2	4,657	0.799	0.572	6.916*	0.206	0.000
Treatment1*Treatment2*Soil	32,657	1.199	0.784	4.174*	0.328	0.000

Table S4.4 Statistics of Fig.4.4, presented are degrees of freedom (df) and F-values folowing a linear mixed model (generalized linear mixed model for leaf yellowness, root darkness and nematode scores) for plant-conditioned soil and un-inoculated soil and a one-way ANOVA (generalized linear model for leaf yellowness, root darkness and nematode score) for sterile soil. *,**,*** indicates significant difference at $P<0.05$, 0.01 or 0.001, respectively. Only plants grown in the diseased soil treatment added to sterile soil had detectable nematode infections. This was not the case for plants grown in sterile soil with the control and Pythium treatments, so there are no one-way ANOVA results for the disease treatments effects. Thus, the F-value in sterile soil is presented as "N.A". Abbreviations of plant species are described in materials and methods.

Species	df	Shoot biomass	Root biomass	Leaf yellowness	Root darkness	Nematode score
AO	8,72	1.096	2.262*	7.730***	0.428	23.049***
BH	8,72	1.285	2.341*	5.564***	0.197	37.27***
FF	8,72	1.078	2.297*	2.504*	0.233	44.124***
GV	8,72	0.716	1.535	8.617***	0.722	20.75***
HL	8,72	0.563	0.831	6.315***	0.551	53.11***
HR	8,72	0.804	2.492*	4.722**	0.523	27.36***
LP	8,72	2.491*	5.628***	7.906***	0.586	40.34***
RA	8,72	1.139	3.645**	5.966***	0.314	30.42***
Un-inoculated	8,72	2.909**	4.111***	8.555***	0.265	28.57***
Sterile soil	2,57	18.22***	4.895*	6.872**	0.310	N.A

Table S4.5 Statistics of Fig.S4.3, presented are degrees of freedom and F-values folowing a linear mixed model (generalized linear mixed model for leaf yellowness). *,**,*** indicates significant difference at $P<0.05$, 0.01 or 0.001, respectively. Abbreviations of disease treatments are described in material and methods.

Treatment	df	Shoot biomass	Root biomass	Leaf yellowness	Root darkness
C-C	8,81	2.450*	1.809	2.265*	0.996
M-C	8,81	1.383	1.137	0.663	0.999
P-C	8,81	1.168	1.567	1.502	0.981
C-P	8,81	1.257	1.062	0.466	0.965
M-P	8,81	0.794	0.484	1.855	0.926
P-P	8,81	1.627	1.111	1.272	0.998
C-D	8,81	1.035	0.796	0.748	0.782
M-D	8,81	1.898	2.073 *	0.778	0.966
P-D	8,81	0.657	0.443	0.787	0.991

Fig.S4.1 Photos of root systems indicative for the root darkness scores at each level.

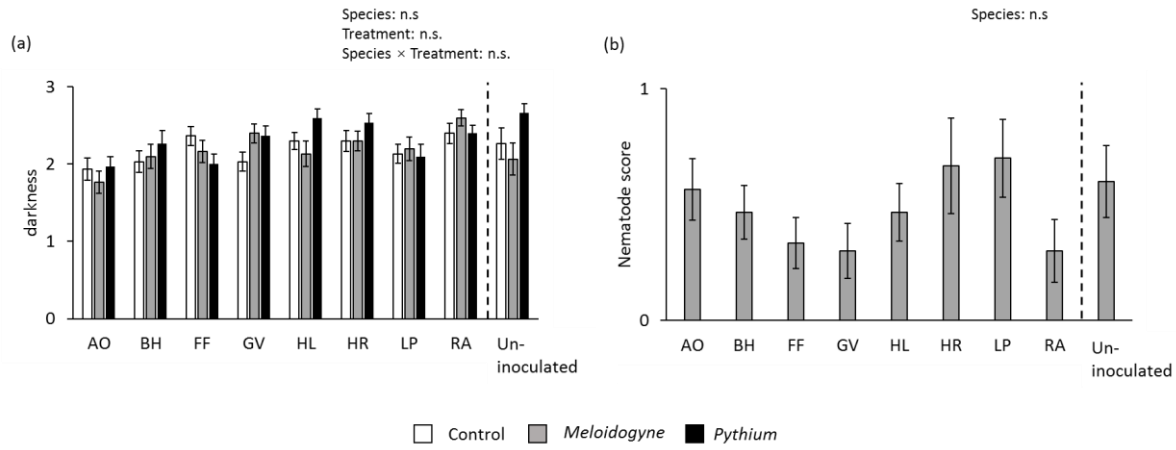

Fig.S4.2 Root darkness (a) and nematode infestation scores (b) of chrysanthemum grown in soil inoculated with plant-conditioned soil and in un-inoculated soil with and without disease treatments after growth cycle 1. White bars indicate root darkness in the control treatment, grey bars indicate the *Meloidogyne* treatment, black bars indicate the *Pythium* treatment. Statistics presented in the upper part of the graph are the results from a generalized linear mixed model for root darkness, and a generalized linear model for nematode score. "n.s." indicates no significant difference.

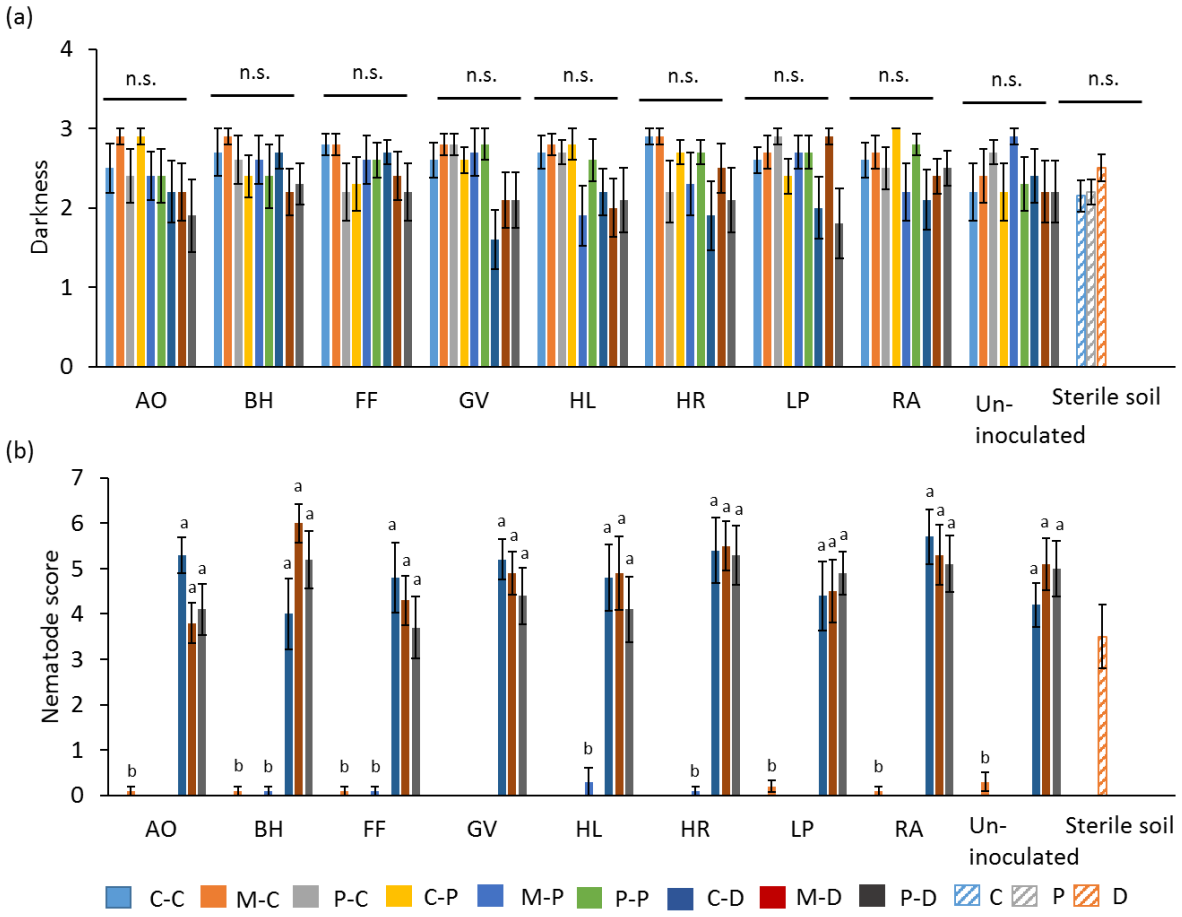

Fig.S4.3 Chrysanthemum root darkness (a) and nematode scores (b) in all soils with and without disease treatments after growth cycle 2. Different colors of the bars indicate different disease treatments. "n.s." indicates no significant difference detected. Within each soil treatment, bars with identical letters are not significantly different.

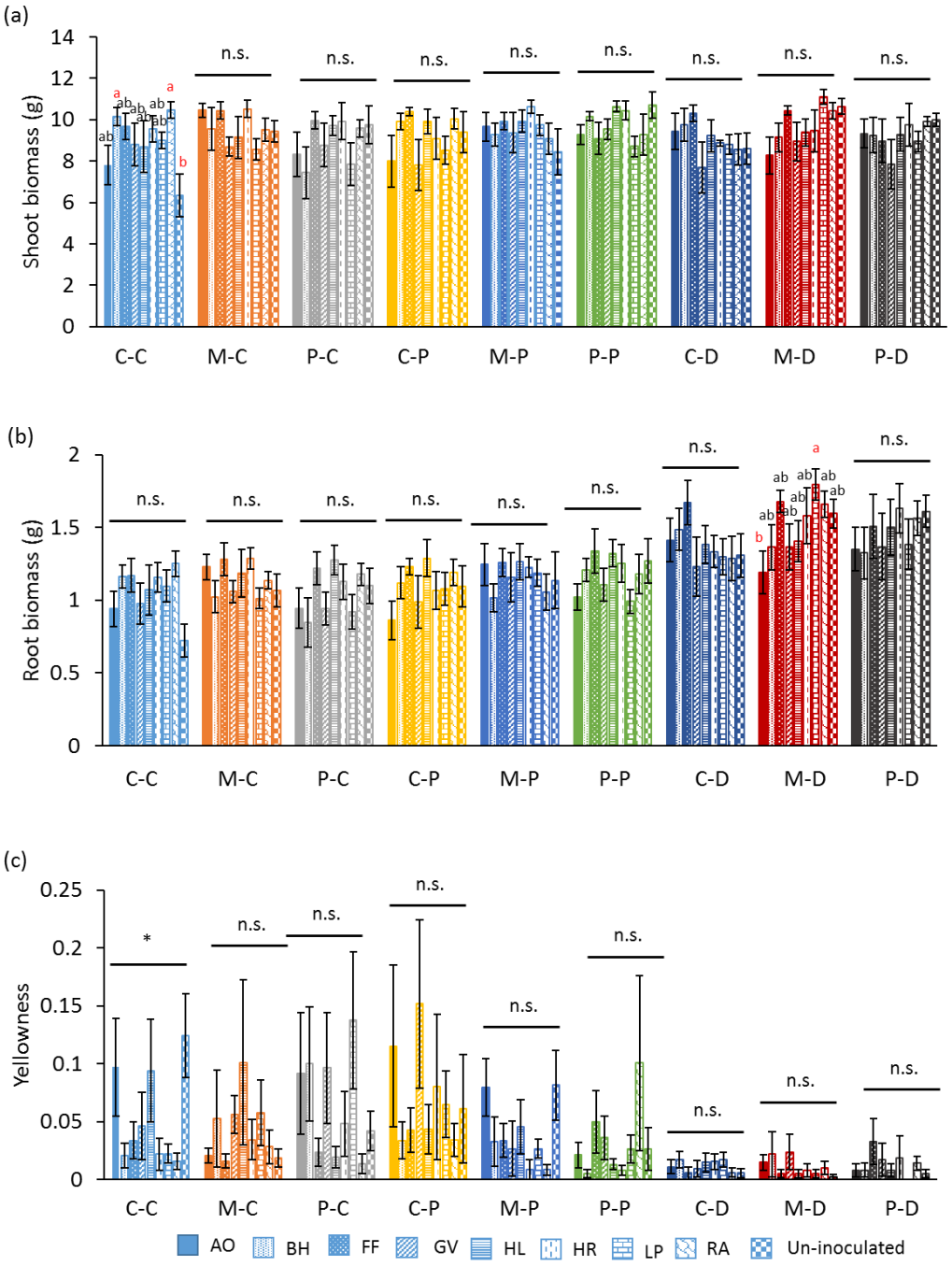

Fig.S4.4 Chrysanthemum shoot biomass (a), root biomass (b) and leaf yellowness (c) in all soil and treatment combinations after growth cycle 2. Presented data are grouped by each disease treatment, in each treatment, different filling patterns of the bars indicate different soil types (plant-conditioned soils or un-inoculated soil). The color of each disease treatment corresponds with Fig.4.3 and Fig.S4.2. "n.s." indicates no significant difference detected. "*" indicates significant difference between soils in the relevant disease treatment ($P < 0.05$), but no significant pairwise comparisons detected. Letters above bars indicate significant differences among soil treatments in that relative disease treatment, bars that are significantly different are highlighted in red. Statistics for each disease treatment are presented in Table S4.5.

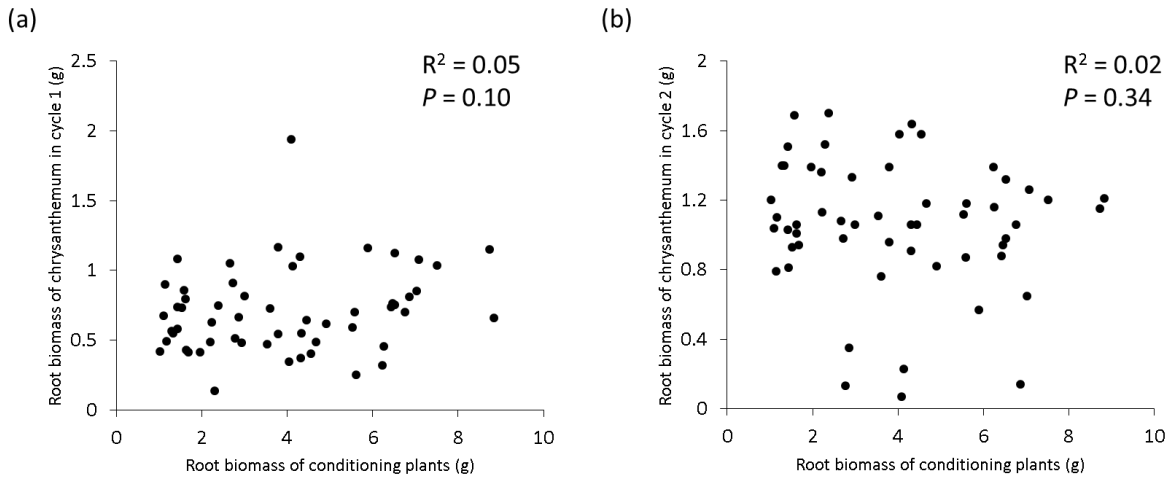

Fig.S4.5 Relationships between root biomass of conditioning plant species and root biomass of chrysanthemum in cycle 1 (a) and cycle 2 (b). R^2 and *P*-values following a linear regression are presented on each panel.

Chapter 5

Plant-soil feedback effects on chrysanthemum growth, susceptibility to aboveground herbivory, and root microbiome composition

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Abstract

Plant-soil feedbacks can be as an important mechanism in driving plant performance in both natural and agricultural systems. However, how and to what extent plant-soil feedbacks can be applied to improve the performance of agricultural crops is currently debated, and whether and how plant-soil feedbacks elucidate changes in the root microbiome of crops is poorly understood. In a two-phase plant-soil feedback experiment, we tested the potential of using plant species and soil from a natural ecosystem to steer the greenhouse soil to become more beneficial for chrysanthemum growth, its root-associated microbiome and aboveground defense. In the conditioning phase, eight wild plant species and chrysanthemum were used to condition either soil collected from a commercial chrysanthemum greenhouse, or soil collected from a natural grassland. In the test phase, the conditioned soils were inoculated in background soil that consisted of live or sterilized greenhouse soil. The effects on chrysanthemum growth, the root-associated microbiome (bacteria and fungi) and the performance of thrips were tested. Inoculation of soil into both live and sterilized background soil significantly influenced the root microbiome of the test plant chrysanthemum. Inoculating natural grassland soil into sterilized greenhouse soil led to higher plant growth, to more complex and connected microbial networks and to a lower abundance of pathogenic fungi in chrysanthemum roots than the other three soil combinations. Soil inoculation did not affect plant shoot biomass when added to live greenhouse soil. However, when chrysanthemum was grown in live greenhouse soil, inoculated with soil from *Lolium perenne*, *Rumex acetosella* and *Festuca filiformis* the microbial diversity in the roots increased, and the relative abundance of pathogenic fungi decreased. The root-associated fungal communities of chrysanthemum grown in live greenhouse soil were dominated by the pathogen *Olpidiomycota* and by *Ascomycota*. The root-associated bacterial communities of chrysanthemum consisted mainly of *Proteobacteria*, *Actinobacteria*, *Patescibacteria*, *Bacteroidetes* and *Cyanobacteria*. The soil type that sustained higher chrysanthemum growth also sustained higher relative abundance of *Chloroflexi*, *Verrucomicrobia*, *Armatimonadetes* and lower relative abundance of *Patescibacteria* in chrysanthemum roots. Out of eight OTUs that were both abundant and highly correlated with plant growth, two OTUs were from *Streptomyces* spp, indicating that this genus may play an important role in chrysanthemum growth. Overall, different soil treatments and the changes in the root microbiome of chrysanthemum did not significantly influence the susceptibility of chrysanthemum to thrips. Our study highlights that inoculation with soil in which first other plant species have been grown alters the root-associated microbiome of chrysanthemum both in sterilized and live background soil, and advances our understanding of the role that plant-soil feedbacks can play in horticulture.

Key words: Root microbiome, Chrysanthemum, Wild plant species, Greenhouse soil, Plant-soil feedback, *Streptomyces*, *Olpidium.*

Introduction

Plant-soil feedbacks are the effects of preceding plants on a succeeding plant by influencing the biotic and abiotic conditions of the soil in which they have grown (Bever et al. 1997; van der Putten et al. 2013). Plant-soil feedback can be an important phenomenon both in natural and in agricultural systems and many plant-soil feedbacks are driven by soil biota (van der Putten et al. 2013; Mariotte et al. 2017). In agriculture, mono-cropping, the continuous cultivation of the same crop, for example, can lead to the build-up of host specialized pathogens in the soil resulting in reduced yields (Mazzoleni et al. 2015; Packer and Clay 2004). Such conspecific plant-soil feedback effects can be avoided by growing other crops in between (*i.e.* crop rotation and cover cropping), because other crop species influence the soil and its microbiome differently (Dias et al. 2013; Kaplan et al. 2018). Recently, several authors have argued that plant-soil feedback effects of wild plant species may be used to improve the soil for the succeeding crop (Vukicevich et al. 2016; Mariotte et al. 2018; Pineda et al. 2017). For example, the grass *Lolium perenne* can increase populations of bacteria that produce antibiotics in the soil, while the grass *Andropogon gerardi* can stimulate the abundance of AM fungi in the soil, which may improve the growth and resistance against soil-borne diseases of the crop that grows later in the soil (Latz et al. 2015; Hetrick et al. 1988). Interestingly, soils from natural ecosystems often contain a diverse soil microbiome with biotic interactions or organisms that could be beneficial in agricultural settings (Mariotte et al. 2017; Morriën et al. 2017). For example, soils from native grasslands suppress the soil pathogen *Rhizoctonia solani* better than soils from agricultural fields (Garbeva et al. 2006), and soils from natural ecosystems typically harbor more diverse communities of entomopathogenic and mycorrhizal fungi than agricultural soils (Meyling et al. 2009; Holland et al. 2016). An important challenge is now to make use of plant-soil feedbacks of plant species and soils from natural ecosystems to enhance the productivity of crops or their resistance against pests and diseases.

Plants shape their rhizosphere microbiomes through a hierarchy of events. First, the bulk soil serves as the "microbial seed bank" (Lennon and Jones 2011). Then, the plant, through rhizodeposition, influences which microbial groups from this reservoir can grow and thrive (Philippot et al. 2013). Some plant species were found to create similar rhizosphere microbiomes in different soils (Miethling et al. 2000; Costa et al. 2006; Wieland et al. 2001). Therefore, it is likely they will also have similar effects on the succeeding plant species when growing in different soils. It is possible to expect that growing non-domesticated plant species in agricultural soil may have the same effects on the soil as growing these plant species in their native soil. However, microbial diversity in agricultural soils is likely to be lower than in natural soils due to the management practices (Mariotte et al. 2017). In addition, microorganisms in natural soils may have long co-evolution histories with wild plant species and this means that they proliferate in natural but not in agricultural soils (Vukicevich et al. 2016). To what extent wild plant

species can be used to change agricultural soils so that the soil becomes more beneficial for crops is still an open question.

The success of introducing a microbial strain into a recipient soil depends at least on four steps: introduction, establishment, growth and spread, and impact (Mallon et al. 2015). The effect of inoculating an entire microbiome is likely to be even more complicated. As different microbes may respond differently to the resident soil. The net impact of the introduced microbiome on the recipient soil will depend, among others, on the adaptation of the introduced microbiome to the new environment and on the resilience of the recipient microbiome to the introduced microbiome (Thomsen and Hart 2018; Mallon et al. 2015). However, studies on disease suppressive soils found that by adding 10% disease suppressive soil to disease conducive soil, the suppressive properties were successfully transferred, although not to the same extent as in 100% disease suppressive soil (Siegel-Hertz et al. 2018; Mendes et al. 2011; Haas and Défago 2005). Hence, an important question is whether and to what extent inoculating soil microbiomes into soils with already existing microbiomes will alter the effects of existing microbiomes on plants.

Soil microbes can play an important role in influencing the chemical composition of the foliage of the plant that grows in the soil and this can subsequently alter the susceptibility of that plant to aboveground pests or diseases (Kos et al. 2015a,b; Badri et al. 2013). The direction of these belowground-aboveground effects may depend on the abundance or composition of microbes in the soil and the plant and pest species tested. Such positive or negative effects of soil microorganisms on plant resistance to aboveground herbivory have been explained by different mechanisms (Kaplan et al. 2018; Pineda et al. 2010). For example, beneficial microbes in the soil, such as mycorrhizal fungi, or plant growth promoting bacteria, can induce systemic resistance in aboveground tissues, which protects the plant against future attack by herbivorous insects (Pineda et al. 2010; Pieterse et al. 2014). However, beneficial microbes may also improve the growth or the nutritional quality of plants, and this can lead to increased levels of aboveground herbivory on the plant (Kaplan et al. 2018; Pineda et al. 2010). Infection by root pathogens which generally hampers plant growth may also, at the same time, induce plant systemic acquired resistance against aboveground herbivory (van Dam 2009; Kammerhofer et al. 2015). The net effect of inoculating a soil community on the susceptibility of a plant to aboveground antagonists will thus be determined by the balance of these opposing forces in the soil and by how this is perceived by the focal plant. A major challenge in agricultural research is now to identify microbiomes that successfully establish after inoculation in soils, and that enhance the growth and hence yield of the crop as well as improve its resistance against pests and diseases.

Here we investigated how inoculation with soils conditioned by eight plant species influences the biomass of chrysanthemum, its root-associated microbiome, and the susceptibility of this crop to an aboveground insect pest. The soil in which the conditioning plants were grown to create the inocula originated either from a natural grassland or was collected from a commercial chrysanthemum greenhouse. Chrysanthemum (*Dendranthema X grandiflora*) is an economically important ornamental in the horticultural industry. Mono-cropping of chrysanthemum in commercial greenhouses leads to a rapid build-up of soil pathogens (Song et al. 2013). To avoid this, the soil is regularly steam-sterilized, a process that kills both detrimental microbes but also beneficial ones. This practice, besides not being sustainable, leaves an empty niche and soil pathogens can easily re-establish in these steamed soils (Thuerig et al. 2009). Previously we showed that inoculating these sterilized soils with live soil in which wild plant species had been grown previously can increase plant growth and reduce the severity of soil pathogens but that the effects depend greatly on the inoculum used (Ma et al. 2017, 2018). In the current study, the plant-conditioned soil inocula were added to either sterilized greenhouse soil, resembling the situation immediately after steaming, or to live greenhouse soil, which was collected after five cycles of chrysanthemum cultivation. We determined the root microbiomes in chrysanthemum plants growing in all combinations of conditioning soil types (natural or greenhouse soil) and background soil types (sterilized or live greenhouse soil). Moreover, we examine whether the susceptibility to Western flower thrips (*Frankliniella occidentalis*), a major aboveground pest of chrysanthemum (Leiss et al. 2009), can be altered by soil inoculation. A better understanding of the role of conditioning plant species, the origin of the soil used for conditioning, and whether the background soil is live or sterilized in influencing the root-associated microbiomes that establish in the crop is important. This can greatly advance our understanding of the potential use of soil inoculations and plant-soil feedbacks in horticulture and may pave the way to new methods that promote crop growth and health (Bakker et al. 2013).

Specifically, we asked five questions, First, will inoculation with soil conditioned by wild plant species enhance chrysanthemum performance compared to inoculation with chrysanthemum-conditioned inocula or un-inoculated soil? Second, will the effects of inoculation with plant-conditioned greenhouse soil resemble the effects of inoculation with native soil when these soils are conditioned by the same plant species? Third, will inoculating soil from different plant species into greenhouse soil positively affect chrysanthemum growth and how does this depend on whether the background soil is sterilized or not? Fourth, how does soil inoculation influence the root-associated microbiome of chrysanthemum? Fifth, which microbial groups in the chrysanthemum root-associated microbiome correlate with chrysanthemum growth and its susceptibility to an aboveground pest?

Materials and methods

Plant and insect material

The focal plant in our study is *Dendranthema X grandiflora* (Ramat.) Kitam. cv. Grand Pink (Chrysanthemum, syn. *Chrysanthemum* X *morifolium* (Ramat.) Hemsl., Asteraceae). Chrysanthemum cuttings were provided by the breeding company FIDES by Dümmen Orange (De Lier, The Netherlands).

A culture of the thrips *Frankliniella occidentalis* was established with a starting colony provided by the company Hazera Seeds (Made, The Netherlands). Thrips were reared for multiple generations on pods of Romano beans (*Vicia faba*) purchased weekly in a local supermarket. Thrips were reared in 0.7 l glass jars with anti-thrips mesh glued to the screw-cap top. To obtain first-instar larvae to use in the experiments, batches of eggs that were laid during a 24 h-period were collected. Thrips were reared in a climate chamber with a 16 h light and 8 h dark photo regime and 25 °C.

Experimental set-up

The experiment consisted of two phases, a conditioning phase and a test phase. In the conditioning phase, eight wild plant species and chrysanthemum were grown individually either in field soil collected from a natural grassland (F) or in greenhouse soil (D) collected from commercial chrysanthemum greenhouse. The conditioning plant species used in this study are four grasses: *Anthoxanthum odoratum*, Poaceae (AO), *Bromus hordeaceus*, Poaceae (BH), *Festuca filiformis*, Poaceae (FF), *Lolium perenne*, Poaceae (LP), four forbs: *Rumex acetosella*, Polygonaceae (RA), *Galium verum*, Rubiaceae (GV), *Achillea millefolium*, Asteraceae (AM), *Tanacetum vulgare*, Asteraceae (TV), and also the focal plant, chrysanthemum (CH). In the test phase, the conditioned soil was used as inoculum (10%) and mixed with either with 90% sterilized greenhouse soil (ST) or 90% live greenhouse soil (D). A chrysanthemum cutting was then planted in each pot, and shoot biomass, the performance of thrips, and the root-associated microbiome were determined. The experimental design is shown in Fig.5.1.

Phase I: Conditioning phase

For the conditioning phase, field soil was collected in (5-20 cm deep) in April 2017 from a semi-natural grassland on former arable land (Mossel, Ede, The Netherlands). The field had been used for agriculture until 1996. The sandy-loam soil was homogenized and sieved (1 cm mesh size) to remove coarse fragments and all macro-arthropods. Greenhouse soil was collected in April 2017 from a commercial chrysanthemum greenhouse, the soil already had five cycles of chrysanthemum cultivation when

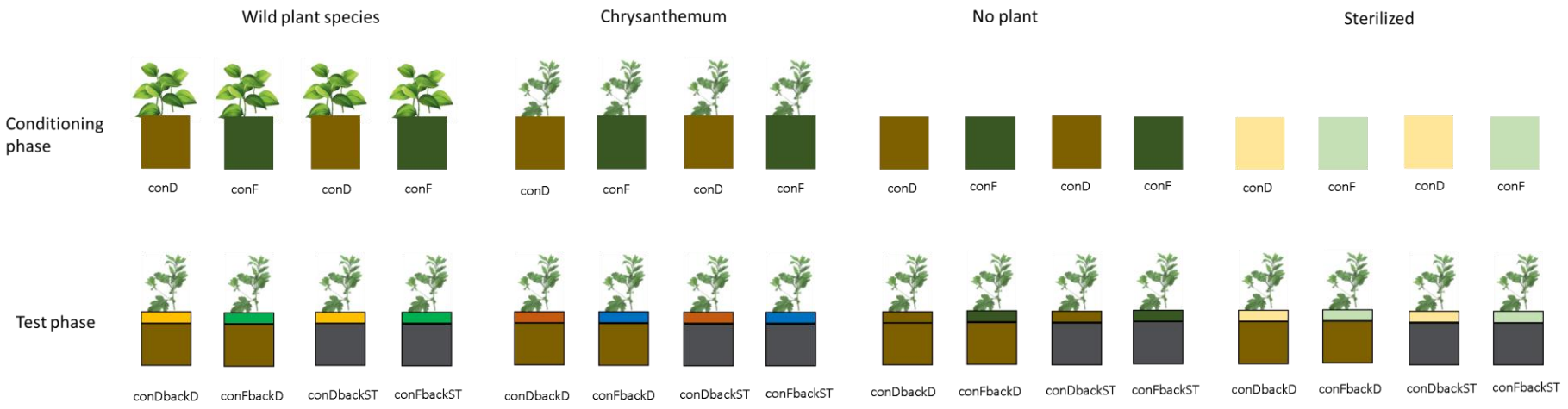


Fig.5.1 Experimental design. For clarity, only one wild plant species out of the eight tested is shown. Details about the conditioning plant species are described in the Materials and Methods section. In the conditioning phase, dark green soil indicates soil collected from a natural grassland; brown soil indicates soil collected from a commericial chrysanthemum greenhouse; light green soil indicates sterilized grassland soil; light yellow soil indicates sterilized greenhouse soil. In the test phase, the colors of inocula correspond to the combination of conditioning plant species and the conditioning soil type. Brown color of background soil indicates the background soil is live greenhouse soil; Grey color of background soil indicates the background soil is sterilized greenhouse soil; "conDbackD" indicates conditioned greenhouse soil with live background soil; "conFbackD" indicates conditioned field soil with live background soil; "conDbackST" indicates conditioned greenhouse soil with sterilized background soil; "conFbackST" indicates conditioned field soil with sterilized background soil.

collected (Brakel, The Netherlands). Pots (13 × 13 × 13 cm) were filled with 1.6 Kg of either field soil or greenhouse soil.

Seeds of the eight wild plant species were obtained from a wild plant seed supplier (Cruydt-Hoeck, Assen, The Netherlands), and were surface sterilized in 3% sodium hypochlorite solution for 1 min, rinsed and germinated on sterile glass beads in a climate chamber at 20 ˚C (16h/8h, light/dark). In each pot, filled with either field soil or greenhouse soil, five one-week-old seedlings were then planted with 10 replicate pots for each species and soil combination. For chrysanthemum, we planted cuttings in the soil and these were then rooted for ten days under thin plastic foil. We also included a set of pots with field soil or greenhouse soil that were not planted but kept in the same greenhouse (no-plant control). In total, the conditioning phase comprised of 200 pots (8 wild plant species × 2 conditioning soil types × 10 replicates + chrysanthemum × 2 conditioning soil types × 10 replicates + no-plant soil × 2 conditioning soil types × 10 replicates). As in a few pots a seedling died after transplantation, the number of seedlings in each pot was reduced to four. All pots were placed randomly in a climate controlled greenhouse with 70% RH, 16 h at 21˚C (day) and 8 h at 16˚C (night). Natural daylight was supplemented by 400 W metal halide lamps (225 μmol $s^{-1}m^{-2}$ photosynthetically active radiation, one lamp per 1.5 m^2). The pots were watered regularly. Ten weeks after transplantation, all conditioning plants were removed from each pot, finer roots were left in the soil as the rhizosphere around the roots may include a major part of the rhizosphere microbial community. The soil from each pot was stored separately in a plastic bag at 4 ˚C for one week until use in the test phase.

Phase II: Test phase

In the test phase, 1 L pots (11 × 11 × 12 cm; length × wide × height) were filled with a homogenized mixture of 10 % soil inoculum (plant-conditioned field soil or plant-conditioned greenhouse soil) and 90 % background soil. The background soil was non-sterilized greenhouse soil or sterilized greenhouse soil. In total, there were 440 pots: [(8 wild plant species + chrysanthemum + no-plant conditioning + sterilized no-plant conditioning) × 2 conditioning soil types × 2 background soil types × 10 replicates]. The soil was sterilized using gamma irradiation (> 25 K Gray, Isotron, Ede, The Netherlands). Two chrysanthemum cuttings (without roots) were planted in each pot as preliminary work showed that not all cuttings establish properly with this method. Prior to planting, the soil in each pot was well-watered and 100 ml half-strength Hoagland nutrient solution was added. The pots were placed on trolleys, each trolley had 48 pots and was tightly covered with a thin transparent plastic film for 10 days to create a closed environment with high humidity that favors rooting. After 10 days, the number of chrysanthemum cuttings in each pot was reduced to one. Plants were fertilized following common grower's practice:

half-strength Hoagland nutrient solution for the first two weeks and single-strength Hoagland solution during the following two weeks. The strength was increased to 1.6 mS/cm EC (electrical conductivity) for the last two weeks. The density of pots on each trolley was reduced two weeks after the start of the second phase to 32 pots per trolley so that there was 10 cm space between each pot. All pots were randomly assigned in the greenhouse with the same conditions as described for the conditioning phase.

Six weeks later, before harvesting, the performance of thrips on a detached plant leaf was measured. The fourth fully-developed leaf (counting form the top) from each plant was detached with a razor blade and placed into a petri-dish. Two one-day old thrips larvae were then placed on the leaf. All petri-dishes were kept in a growth chamber (24°C, 16h day 8h night) and their positions were randomly rotated several times a week. Ten days later, the life stages (pupa, larva or adult) of the thrips in each petri-dish was recorded. Adult thrips were frozen, and their gender and body length (mm) were recorded using a stereo microscope. The damage area on each leaf was recorded using transparent paper with a square millimeter raster and counting by eye the number of mm^2 showing silver leaf damage. All detached leaves were oven-dried (60 ˚C for 3 days) and the weight of the leaf was added to the total shoot biomass of the corresponding plant. After clipping the test leaf, plants were harvested. Each plant was clipped at soil level, and shoot biomass was oven-dried (60 ˚C for 3 days) and weighed. Roots were washed over a sieve (2 mm mesh) using tap water until there was no visible soil attached to the roots. All root samples were then freeze dried and stored at -20 ˚C to be used for root-associated microbiome analysis.

Microbial DNA extraction

For each treatment, replicate numbers 1 to 5 were used for DNA extraction. In total, root microbiomes of 220 samples were analyzed. Before extracting DNA, all freeze-dried roots were ground into powder using TissueLyser II, QIAGEN. DNA was extracted from 40 mg powdery freeze-dried root using the FastDNA SPIN Kit (MP Biomedicals, Solon, OH, USA) following the manufacturer's protocol. The DNA quantity was measured using a Nanodrop spectrophotometer (Thermo Scientific, Hudson, NH, USA). All samples yielded between 100-400 ng/nl of DNA. We then carried out PCR using primers ITS4ngs and ITS3mix targeting the ITS2 region of fungal genes (Tedersoo et al. 2015) and the primers 515FB and 806RB (Caporaso et al. 2012) targeting the V4 region of the 16Sr RNA for bacteria. PNA were used to block plant DNA (Lundberg et al. 2013). We used the Phusion Flash High-Fidelity PCR Master Mix (Thermo Scientific, Hudson, NH, USA). The cycling conditions for bacteria were 98 °C for 3 min followed by 25 cycles of 98 °C for 15 s, 55 °C for 15 s and 72 °C for 30 s. The cycling conditions for fungi were 98 °C for 3 min followed by 30 cycles of 98 °C for 15 s, 55 °C for 15 s and 72 °C for 30 s. Final extension for both was 72 °C for 3 min. Both a positive (mock community consisting of 10

fungal strains) and a negative control (water) were included in the amplification steps. Presence of PCR product was verified using agarose gel electrophoresis. The PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter). Adapters and barcodes were added to samples using Nextera XT DNA library preparation kit sets A-C (Illumina, San Diego, CA, USA). The final PCR product was purified again with AMPure beads, checked using agarose gel electrophoresis and quantified with a Nanodrop spectrophotometer before equimolar pooling. The final libraries of bacteria consisted of 220 sample, and fungi consisted of 219 samples (one failed) (supplementary information). Both fungi and bacteria were sequenced in 4 separate MiSeq PE250 runs. A mock community was included to compare between runs. The samples were sequenced at McGill University and Génome Québec Innovation Centre (Canada).

The data for bacteria was analyzed using an in-house pipeline (de Hollander 2017). The SILVA database was used to classify bacteria. Fungal data was analysed using the Pipits pipeline (Gweon et al. 2015). The UNITE database (Abarenkov et al. 2010) was used for identification of fungi and the ITSx extractor was used to extract fungal ITS regions (Nilsson et al. 2010). FUNGuild (Nguyen et al. 2016) was used to classify fungal OTUs into potential functions. The OTUs that could be classified were grouped into saprophytes, AMF, plant pathogens, plant symbionts, plant endophytes, and rest (Ectomycorrhizal, fungal/animal/unidentified plant pathogens). Standardization of the sequencing data is presented in the Supplementary Information.

Statistical analysis

The effects of conditioning (all inocula treatments, including sterilized inocula, no-plant conditioning inocula), conditioning soil type and background soil type on plant shoot biomass, leaf silver damage area and body length of thrips were examined using a linear mixed model. In the model, inoculum type, conditioning soil type and background soil type were defined as fixed factors, and soil replicate as random factor. Tukey *post-hoc* tests were used for pairwise comparisons between conditioning and background soil type combinations. For each conditioning soil and background soil type combination, a one-way ANOVA was used to test the overall differences between inocula. For each soil type, we used three different controls: sterilized no-plant inocula, no-plant inocula and chrysanthemum conditioned inocula. *Post hoc* Dunnet tests were used to compare each inoculum effect with the controls.

Analysis of sequencing data: Permutational multivariate analysis of variance (PERMANOVA) was used to test whether bacterial and fungal communities were significantly influenced by inoculum type,

conditioning soil type and background soil type. Non-metric multidimensional scaling (NMDS) based on Bray-curtis distances was used to visualize the similarities between the four conditioning and background soil combinations. A cluster analysis based on Ward's method (Ward 1963) was used to explore Bray-curtis based distances between each treatment.

Network analysis: Co-correlation network analysis was performed to visualize the interactions among microbial taxa. Spearman Rank correlations were used to determine non-random co-occurrences. For this, only dominant OTUs which occurred in more than 90% of the samples were included. Correlations among OTUs with statistically significant (P<0.01 after Bonferroni correction) and a magnitude of >0.7 or <-0.7 were included in the network analysis (Barberán et al. 2012). Each node in the network represents an individual OTU, whereas the edges represent significantly positive or negative correlations between nodes (Barberán et al. 2012). The network properties and topologies were measured based on the number of nodes, edges, average degree and average clustering coefficient. The visualization and properties measurements were calculated with the interactive platform Gephi.

Inverse Simpson diversity was calculated for both bacteria and fungi communities. Pearson correlations were used to determine the correlations between bacterial and fungal diversity with shoot biomass, leaf silver damage area and thrips body length. To explore whether the relative abundance of particular bacterial or fungal OTU was related to shoot biomass, leaf silver damage area, or body length of thrips, Pearson correlations were used. After Bonferroni correction, correlations with P<0.05 were considered as significantly correlated OTUs. Explained variance (R) was always higher than 38% for all selected OTUs. Among the chrysanthemum growth-correlated OTUs, OTUs with average relative abundance higher than 1% were selected for further analysis of the treatments effects.

The overall effects of conditioning plant species (including sterilized inocula and no-plant conditioning inocula), conditioning soil type, and background soil type, on the relative abundance of bacterial and fungal phyla of chrysanthemum roots were tested using a linear mixed model. The bacterial phyla which had on average a relative abundance of less than 0.001% were grouped into "low abundance". In the model, inoculum type, conditioning soil type and background soil type were used as fixed factors, soil replicate was used as random factor. For each soil type, a one-way ANOVA was used to test the overall differences between inocula. Then a *post hoc* Dunnet test was used to compare each inoculum effect with those of controls (sterilized inocula, no-plant conditioning inocula, and chrysanthemum conditioned inocula). The same analyses were also performed to test the effects of inoculum type, conditioning soil type and background soil type on bacterial diversity, fungal diversity, OTUs that both

highly correlated with plant shoot biomass and had an average abundance higher than 1%, and to compare the functional classification of fungal groups.

Results

Conditioning plant species and soil type effects on chrysanthemum growth and thrips performance

Overall, chrysanthemum shoot biomass was higher in sterilized background soil than in live background soil. Inocula from field soil were better for chrysanthemum growth than inocula from greenhouse soil when the background soil was sterilized, while there were no significant differences between these two conditioning soil types when the background soil was live greenhouse soil. Body length of female thrips was higher with inocula from field soil than with inocula from greenhouse soil (Table 5.1, Fig.5.2). Body length of male thirps and leaf silver damage area were not significantly influenced by any treatments (Table 5.1, Fig.5.2). The effects of inoculation depended on the combination of conditioning soil type and background soil type. For inocula from field soil with live background soil, inoculation with soil from *Festuca filiformis* resulted in higher plant shoot biomass than inoculation with chrysanthemum-conditioned soil. Inoculating sterilized conditioned greenhouse or field soils into sterilized background soil, resulted in the highest shoot biomass of chrysanthemum (Fig.5.2a).

Conditioning plant species and soil type effects on the diversity and community structure of the root microbiome

The composition of the root-associated bacterial community and bacterial diversity were significantly influenced by conditioning plant species, conditioning soil type and background soil type (Table 5.1, 5.2). Bacterial diversity in chrysanthemum roots was higher in sterilized background soil than in live background soil (Table 5.1, Fig.5.3). There were significant two way and three way interactions on the composition of root-associated bacterial communities (Table 5.2). The composition of root-associated fungi and fungal diversity were not significantly influenced by conditioning plant species, but significantly differed among soil types and there were significant interaction effects (Table 5.1, 5.2). Inoculating conditioned field soils into sterilized background soil led to significantly higher chrysanthemum root fungal diversity than inoculation of conditioned greenhouse soils into sterilized background soil (Table 5.1, Fig.5.3).

Table 5.1 Effects of conditioning (all soil treatments, including sterilized no-plant inocula, no-plant inocula), conditioning soil type and background soil type on chrysanthemum shoot biomass, leaf silver damage area, body length of female and male thrips, bacterial and fungal diversity. "consoil" indicates conditioning soil type, "backsoil" indicates background soil type. Presented are F-values following linear mixed model tests, T-values are presented for pairwise comparisons between soil types. "D,D" indicates conditioned greenhouse soil with live background soil. "D,ST" indicates conditioned greenhouse soil with sterilized background soil. "F,D" indicates conditioned field soil with live background soil. "F,ST" indicates conditioned filed soil with sterilized background soil. *,**,*** indicate significant differences at $P<0.05$, 0.001 and 0.0001, respectively. Contrasts following a non-significant conditioning soil type and background soil type interaction were not calculated.

	Shoot biomass		Silver damage area		Female body length		Male body length		Bacterial diversity		Fungal diversity	
	df	F value	df	F value	df	F value	df	F	df	F value	df	F value
Inocula	10,180	2.11*	10,180	1.05	10,141	0.37	10,142	0.91	10,80	2.14*	10,83	0.73
Consoil	1,180	52.85 ***	1,180	0.14	1,141	4.74*	1,142	0.03	1,80	1.53	1,83	0.12
Backsoil	1,216	554.92 ***	1,210	0.95	1,53	1.56	1,142	0.76	1,87	29.65***	1,83	0.54
Consoil × Backsoil	1,216	93.27 ***	1,210	0.10	1,53	3.26	1,142	1.78	1,87	0.13	1,83	5.48*
D,D - F,D		1.29										NA
D,D - D,ST		-9.83 ***										1.84
D,D - F,ST		-20.75 ***										-1.19
F,D - D,ST		-10.51 ***										NA
F,D - F,ST		-23.48 ***										NA
D,ST - F,ST		-11.53 ***										-2.82*
Inocula × Consoil	10,180	1.56	10,180	1.31	10,141	0.39	10,142	0.83	10,80	1.24	10,83	1.36
Inocula × Backsoil	10,216	7.89 ***	10,210	0.88	10,53	0.52	10,142	1.00	10,87	0.72	10,83	0.39
Inocula × Consoil × Backsoil	10,216	1.48	10,210	1.10	10,53	0.69	9,142	0.25	10,87	1.21	10,83	0.56

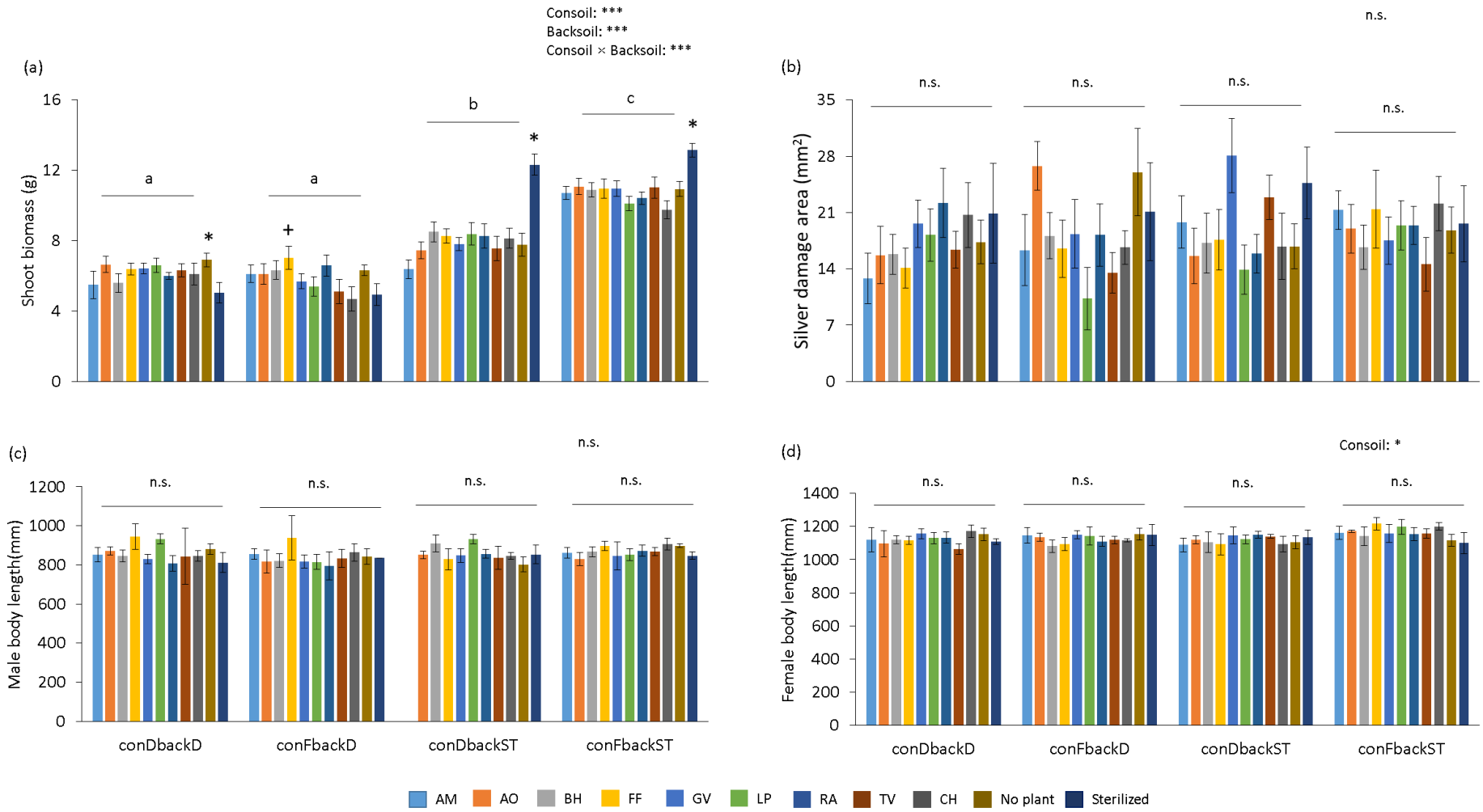


Fig.5.2 Chrysanthemum shoot biomass (a), leaf silver damage area (b), body length of male thrips (c) and body length of female thrips (d) in different conditioning and background soil type combinations conditioned by wild plant species, chrysanthemum, no-plant conditioning and sterilized no-plant conditioning soils. In each bar plot, statistics of the overall effects are presented in the upper part of the figure, only significant effects are shown. "*" above bars (not for the bar of "sterilized no-plant inocula") indicate significant differences compared with sterilized no-plant inoculum in that conditioning and background soil combination. "*" above the bar for sterilized no-plant inoculum

indicates that the sterilized inoculum is significantly different from all the other bars in that soil combination. "+" above bar indicates significant difference compared with chrysanthemum soil inoculum. Letters above each group of bars represent whether the groups differences significantly. "n.s." indicates there were no significant differences between groups. "conDbackD" indicates conditioned greenhouse soil with live background soil; "conFbackD" indicates conditioned field soil with live background soil; "conDbackST" indicates conditioned greenhouse soil with sterilized background soil; "conFbackST" indicates conditioned field soil with sterilized background soil. Full names of the plant species are described in the materials and methods section, "No-plant" in the legend indicates no-plant conditioned inocula, "Sterilized" in the legend indicates sterilized no-plant soil inocula.

Table 5.2 Effects of conditioning (all soil treatments, including no-plant inocula and sterilized no-plant inocula), conditioning soil type and background soil type on the composition of bacterial and fungal OTUs. Presented are degree of freedom (df), F-value and explained R^2 following a PERMANOVA test. *,**,*** indicates significant differences at P<0.05, 0.01 and 0.001, respectively.

	Bacteria			Fungi		
	df	F value	R^2	df	F value	R^2
Inocula	10,163	2.42***	0.06	10,83	1.28	0.08
Consoil	1,163	36.19***	0.10	1,83	9.23***	0.06
Backsoil	1,163	74.85***	0.20	1,83	5.73***	0.04
Inocula × Consoil	10,163	2.18***	0.06	10,83	1.28	0.08
Inocula × Backsoil	10,163	1.66***	0.04	10,83	1.13	0.07
Consoil × Backsoil	1,163	20.09***	0.05	1,83	5.33***	0.03
Inocula × Consoil × Backsoil	10,163	1.50**	0.04	9,83	1.38*	0.08

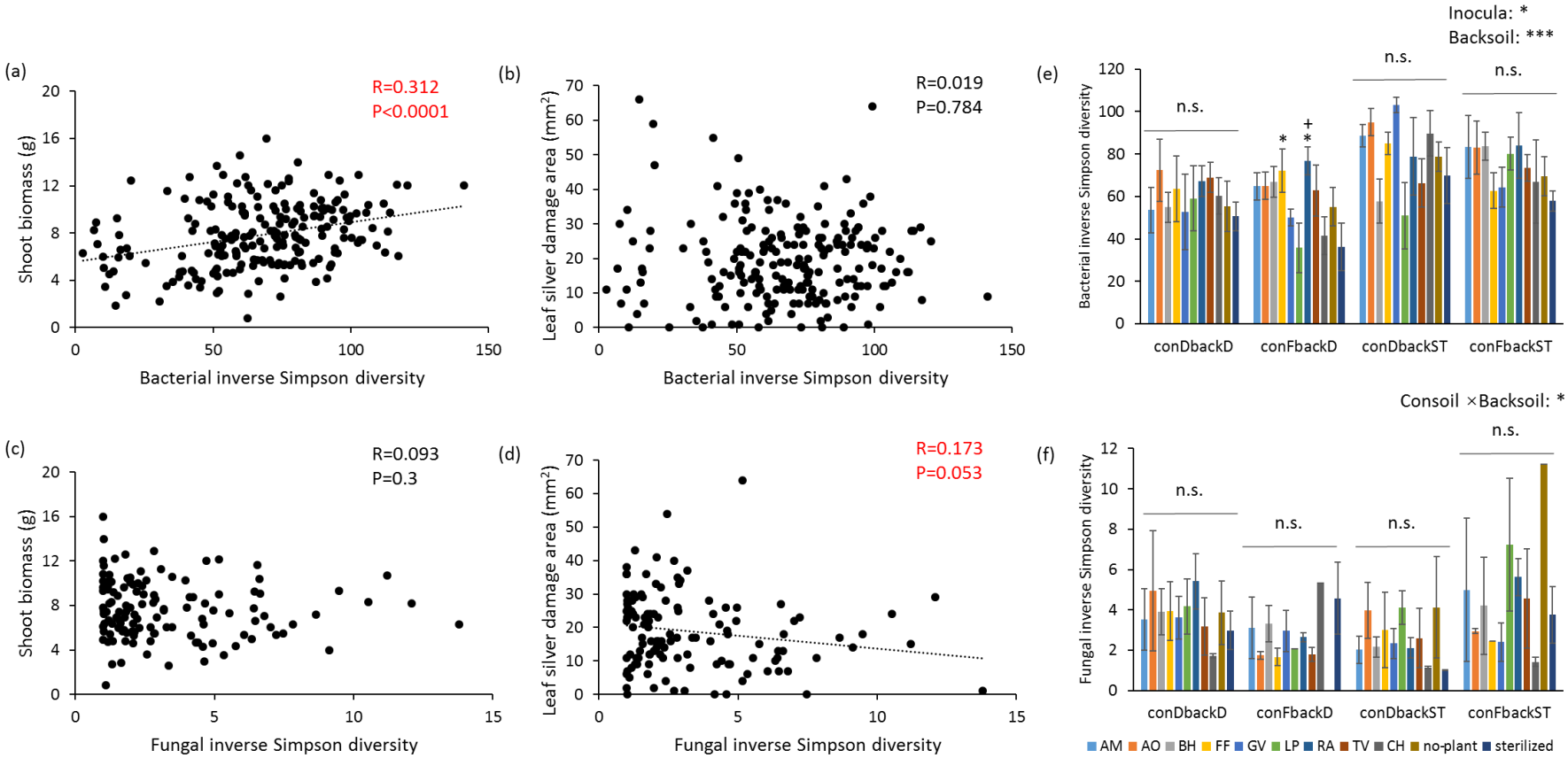


Fig.5.3 Relationships between root-associated bacterial and fungal diversity with chrysanthemum shoot biomass (a,c), leaf silver damage area (b,d) and bacterial and fungal diversity in different soil treatments (e,f). In each bar plot, statistics of the overall effects are presented in the upper part of the figure, only significant effects are showed. "*" above bar indicates significant difference compared with sterilized no-plant inoculum in that relative soil type. "+" above bar indicates significant difference compared with chrysanthemum-conditioned inoculum in that relative soil type. "n.s." indicates no significant differences between conditioning treatments in the relative soil type. "conDbackD" indicates conditioned greenhouse soil with live background soil; "conFbackD" indicates conditioned field soil with live background soil; "conDbackST" indicates conditioned greenhouse soil with sterilized background soil; "conFbackST" indicates conditioned field soil with sterilized background soil. "no-plant" indicates no-plant conditioning. "sterilized" indicates sterilized no-plant inocula.

Overall, bacterial diversity positively correlated with chrysanthemum shoot biomass, while there were no correlations between bacterial diversity and other plant parameters, or between fungal diversity and any plant parameters (Fig.5.3, Fig.S5.2). For the conditioned field soil with live background soil combination, inoculation with *Festuca filiformis* and *Rumex acetosella* soil led to higher chrysanthemum root bacterial diversity than inoculation with sterilized soil. Inoculation with soils conditioned by *Rumex acetosella*, resulted in the same effect when compared with chrysanthemum-conditioned soil (Fig.5.3e).

The NMDS and Ward's cluster analysis revealed a distinctive separation between bacterial communities from field and greenhouse soil inocula, when the background soil was sterilized. There was greater overlap between bacterial communities originating from the different conditioning soils when the background consisted of live soil (Fig.5.4a,c). There was no clear separation in fungal communities between the conditioning and background soil type combinations (Fig.5.4b,d). The effects of conditioning plant species on the community structure of the bacterial and fungal communities in the different treatments was not consistent (Fig.5.4c,d). Network analysis showed that microbiomes from conditioned field soils added to sterilized background soil had a more complex soil microbial network than the other three soil combinations. Microbiomes belonging to the combination conditioned field soils added to sterilized backgrounds soil, were characterized by higher numbers of nodes, edges and connections per node (average degree) (Fig.5.5, Table 5.3).

Conditioning plant species and soil type effects on the composition of root-associated bacterial and fungal communities

In the chrysanthemum root associated microbiome, *Proteobacteria*, *Actinobacteria*, *Patescibacteria*, *Bacteroidetes*, *Cyanobacteria* and *Planctomycetes* were the most abundant bacterial phyla (Fig.5.6a). Inoculation with greenhouse soils led to a higher relative abundance of *Proteobacteria* in the root associated microbiome of chrysanthemum than inoculation with field soils (Fig.5.6a, Table S5.1). In sterilized background soil, the relative abundance of *Patescibacteria* was fewer, and the relative abundance of *Actinobacteria*, *Chloroflexi*, *Verrucomicrobia*, *Armatimonadetes* higher in roots compared to live background soil. Except for *Actinobacteria*, addition of conditioned field soils to sterilized background soil made these patterns stronger (Table S5.1, Fig.5.6). The relative abundances of *Bacteroidetes*, *Acidobacteria* and *Firmicutes* changed but only in sterilized background soil inoculated with field soil, which led to lower relative abundances of *Acidobacteria*, and higher relative abundances of *Bacteroidetes* and *Firmicutes* in chrysanthemum roots than in the other three soil combinations.

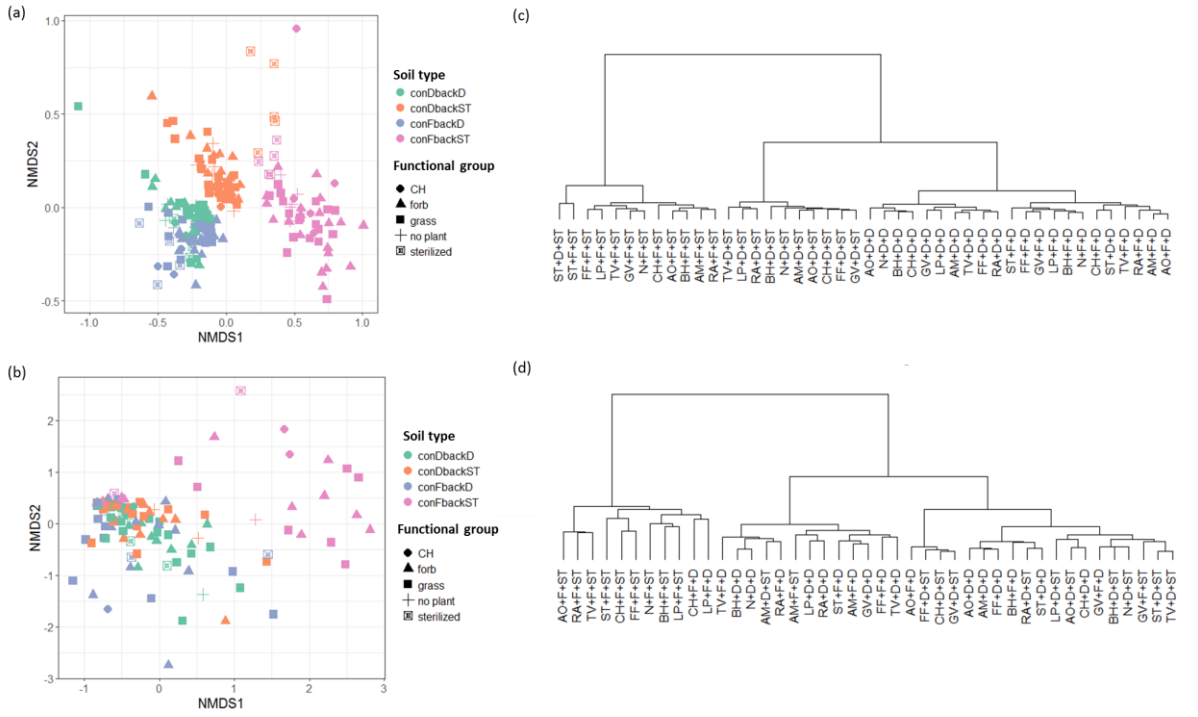


Fig.5.4 Non-metric multidimensional scaling (NMDS) plot performed on taxonomic profile (OTU level for 16s and ITS DNA) of root-associated bacteria (a) and fungi (b), and the hierarchical cluster analysis of bray-curtis similarities between each treatment on root-associated bacteria (c) and fungi (d). For NMDS plots, the four types of conditioning soil and background soil combinations are highlighted in different colors. The functional groups of conditioning plant species are presented by different shapes. "conDbackD" indicates conditioned greenhouse soil with live background soil; "conFbackD" indicates conditioned field soil with live background soil; "conDbackST" indicates conditioned greenhouse soil with sterilized background soil; "conFbackST" indicates conditioned field soil with sterilized background soil. "no plant" indicates no-plant conditioning. "sterilized" indicates sterilized no-plant inocula. In cluster analysis, the names of treatments are consisted of conditioning plant species identity + conditioning soil type + background soil type. The abbreviations of conditioning plant species are describes in material and methods. "ST" indicates sterilized inocucla. "N" indicates no-plant conditioning inocula. "D" indicates greenhouse soil, "F" indicates grassland soil. "ST" indicates sterilized soil.

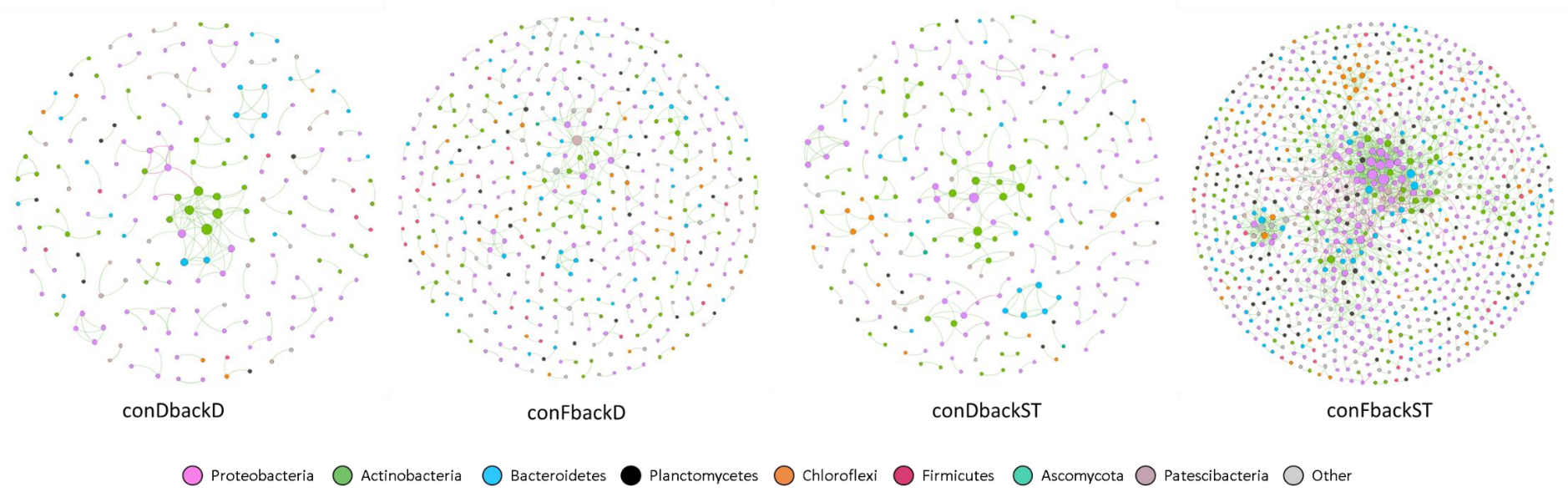


Fig.5.5 Network co-occurrence analysis of chrysanthemum root-associated microbial communities in the four types of conditioning and background soil combinations. A connection stands for a Spearman Rank correlation with magnitude > 0.7 (both positive and negative) that is statistically significant ($P < 0.05$ with Bonferroni correction). Red edges indicate negative correlations, green edges indicate positive correlations. Each node represents an OTU, and the size of the node is proportional to its number of connections (*i.e.* degree). Each node was colored at phylum level. "conDbackD" indicates conditioned greenhouse soil with live background soil; "conFbackD" indicates conditioned field soil with live background soil; "conDbackST" indicates conditioned greenhouse soil with sterilized background soil; "conFbackST" indicates conditioned field soil with sterilized background soil.

Table 5.3 Topological properties of co-occurrence network of root-associated microbial communities in four soil types. Networks are in Fig.5.5.

Network Properties	conDbackD	conDbackST	conFbackD	conFbackST
Number of nodes[a]	193	276	453	978
Number of edges[b]	172	244	365	1676
Average degree[c]	1.782	1.768	1.611	3.427
Average clustering coefficient[d]	0.61	0.593	0.29	0.313

[a] Microbial taxon (based on OTU) with at least one significant (P<0.01) and strong (Spearman Rank correlations >0.7 or <-0.7) correlation.

[b]Number of connections/correlations obtained by Spearman Rank correlation analysis.

[c]The acerage number of connections per node in the network, i.e. the node connectivity (Gephi).

[d]How nodes are embedded in their neighborhood and the degree to which they tend to cluster together (Gephi).

The differences in bacterial phylum composition between different plant conditioned inocula were mainly due to the distinctive phylum composition in 100% sterilized soil. Inoculation of sterilized soil into sterilized background soil led to a lower relative abundance of *Actinobacteria*, *Acidobacteria* and a higher relative abundance of *Cyanobacteria*, *Chloroflexi*, and *Armatimonadetes* in the root microbiome compared to inoculation of plant-conditioned inocula (Fig.5.6a,b). For conditioned greenhouse soil added to sterilized background soil, inoculation of *Galium verum* soil led to lower relative abundance of *Actinobacteria* and higher relative abundance of *Cyanobacteria* in the root microbiome of chrysanthemum than chrysanthemum-conditioned soil (Fig.5.6a). *Rumex acetosella* conditioned field soil added to live background soil resulted in a relatively higher abundance of *Cyanobacteria* in the chrysanthemum root microbiome than with sterilized inocula, no-plant conditioned inocula and chrysanthemum conditioned inocula (Fig.5.6a). *Lolium perenne* conditioned field soil added to sterilized background soil, resulted in a higher relative abundance of *Verrucomicrobia* than the three control treatments (Fig.5.6b).

The fungal community in chrysanthemum roots consisted mainly of *Olpidiomycota* and *Ascomycota. Olpidiomycota* is a phylum that consists of plant pathogenic fungi (Fig. 5.6c). The relative abundance of *Olpidiomycota* in chrysanthemum roots was lower with conditioned field inocula and sterilized background soil than in the other three conditioning and background soil combinations. Addition of conditioned greenhouse soil to sterilized background soil increased the relative *Olpidiomycota* abundance in roots relative to adding the same inocula into live background soil (Table S5.2, Fig.5.6c). The relative abundance of *Ascomycota*, *Mortierellomycota* and *Mucoromycota* was significantly increased after inoculation of conditioned field soil into sterilized background soil compared to the other three soil combinations (Table S5.2, Fig.5.6c).

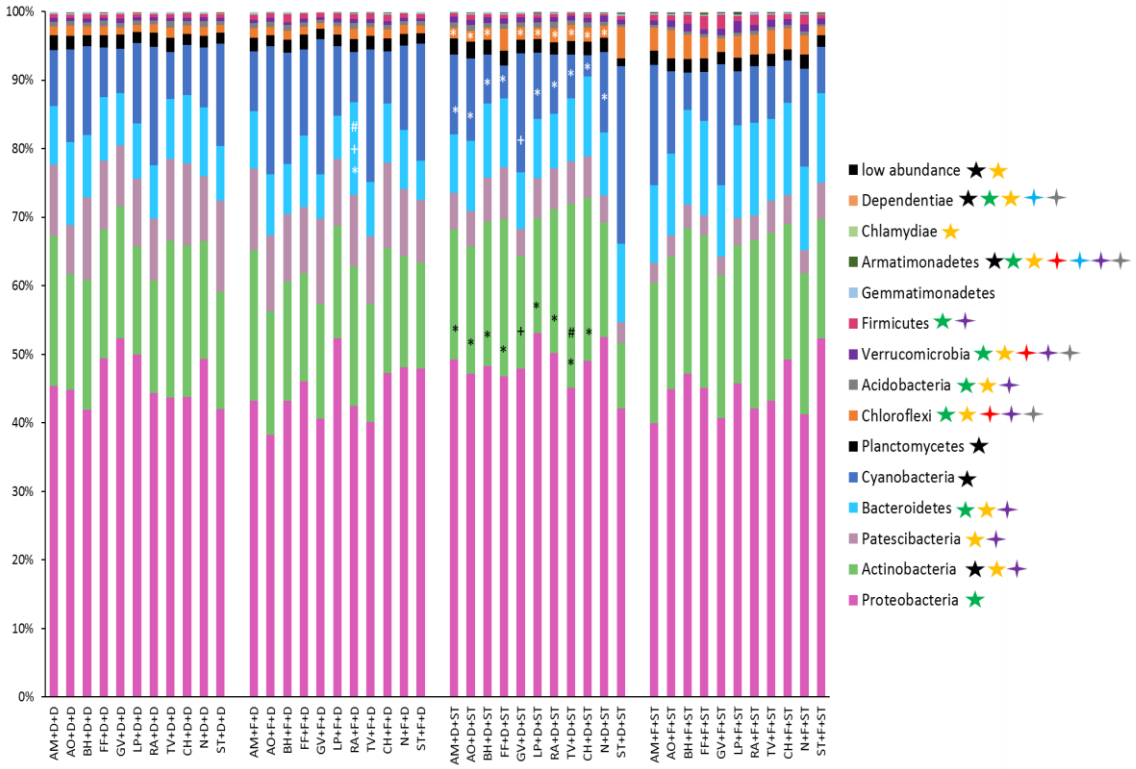
Roots of chrysanthemum growing in greenhouse soil inocula and sterilized background soil that were conditioned by *Lolium perenne*, *Anthoxanthum odoratum* and *Achillea millefolium* had lower relative abundance of *Olpidiomycota* are higher relative abundance of *Ascomycota* (except for *Achillea millefolium*) than roots growing in 100% sterilized soil (Fig.5.6c). For *Lolium perenne* inoculation, the same effect was also significant when compared with chrysanthemum conditioned inocula (Fig.5.6c).

When classifying root-associated fungi based on their functional groups, the responses of pathogenic fungi to conditioning plant species and soil treatments were the same as for *Olpidiomycota*, because *Olpidiomycota* contributed substantially to the abundance in this group (Table 5.4, Fig.5.7). Saprotrophic fungi and plant symbiotic fungi had higher relative abundances in treatments consisting of conditioned field inocula and sterilized background soil than in the other three soil combinations (Table 5.4, Fig.5.7).

Conditioning plant species and soil type effects on the microbial taxa that correlate highly with plant performance

After Bonferroni correction, only bacterial OTUs significantly correlated with plant shoot biomass. No bacterial or fungal OTUs correlated with leaf silver damage area or thrips body length. OTUs that were highly correlated with plant shoot biomass are shown in Table S5.3. There were eight OTUs that correlated with chrysanthemum growth and that had an average abundance of more than 1%: *Streptomyces* 1 (OTU-5), Unidentified *Saccharimonadales* 1 (OTU-9), Unidentified *Micromonosporaceae* (OTU-15), Unidentified *Saccharimonadales* 2 (OTU-23) and *Glycomyces* (OTU-29)

(a)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Armatimonadetes
Gemmatimonadetes
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Chloroflexi
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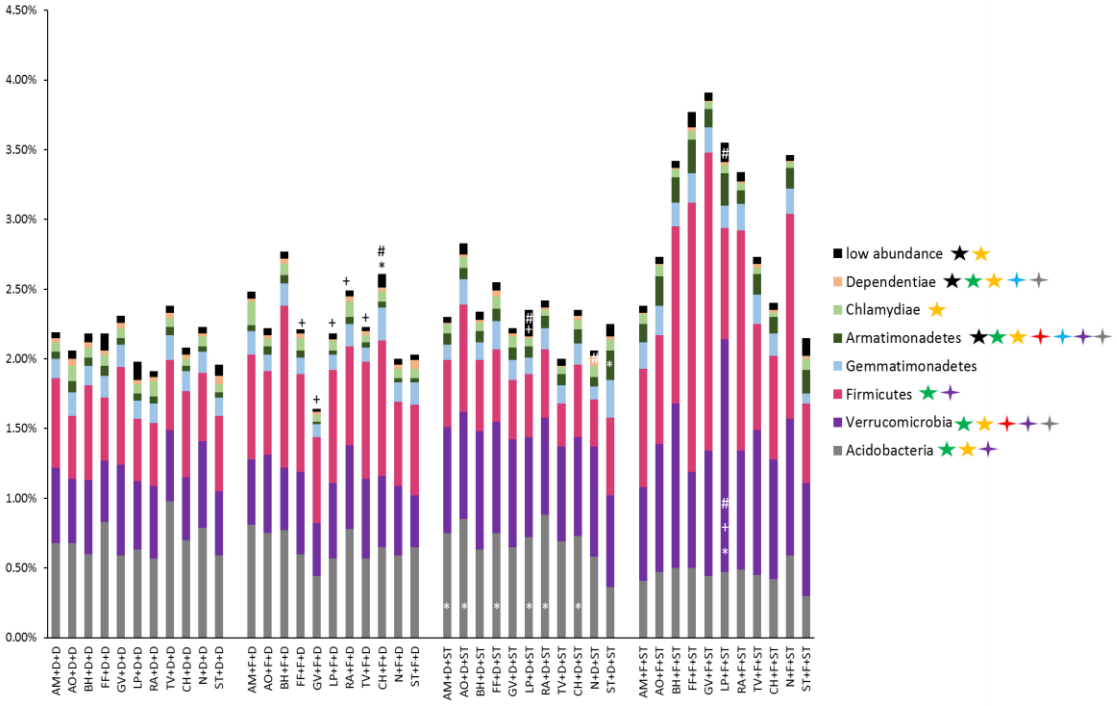
Cyanobacteria
Bacteroidetes
Patescibacteria
Actinobacteria
Proteobacteria

(b)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BH+F+ST
FF+F+ST
GV+F+ST
LP+F+ST
RA+F+ST
TV+F+ST
CH+F+ST
N+F+ST
ST+F+ST
low abundance
Dependentiae
Chlamydiae
Armatimonadetes
Gemmatimonadetes
Firmicutes
Verrucomicrobia
Acidobacteria

(c)

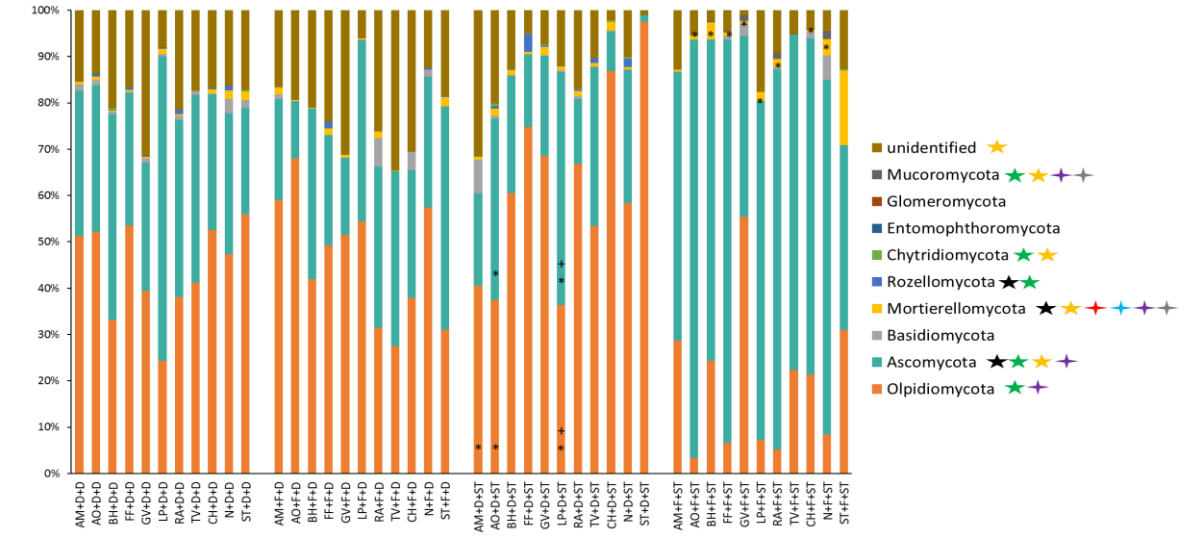

Fig.5.6 The relative abundance of bacterial phyla (a,b) and fungal phyla (c) in each soil treatment. Fig.5.6a and b both show bacterial phyla composition, Fig.5.6b shows the relative low abundance phyla which are not visible in Fig.5.6a. Five-point stars following the legend of each phylum represent significant effects of factors and four-point stars represent significant interactions between factors following linear mixed model. Black stars indicate significant effects of conditioning plant species; Green stars indicate significant effects of conditioning soil type; Yellow stars indicate significant effects of background soil type; Red stars indicate significant interactions between conditioning plant species and conditioning soil type; Blue stars indicate significant interactions between conditioning plant species with background soil type; Purple stars indicate significant interactions between conditioning soil type and background soil type; Grey stars indicate significant interactions between all three factors. In each soil type, "*" indicates significant difference compared with sterilized soil inocula; "+" indicates significant difference compared with chrysanthemum-conditioned inocula; "#" indicates significant difference compared with no-plant conditioned inocula; Name of each bar is labeled as conditioning plant species + conditioning soil type + background soil type, in which "N" = no-plant, "ST" = sterilized, "F" = field soil, "D" = greenhouse soil.

were negatively correlated with chrysanthemum shoot biomass, and their explained variance (R) of plant shoot biomass was 0.59, 0.41, 0.41, 0.57 and 0.42, respectively (Fig.S5.3). *Paenarthrobacter* (OTU-14), *Streptomyces* 2 (OTU-10) and *Rhizobium* (OTU-13) were positively correlated with shoot biomass, and their explained variance of plant shoot biomass was 0.49, 0.46 and 0.51, respectively (Fig.S5.3).

Table 5.4 The effects of conditioning plant species (all soil treatments, including no-plant conditioned and sterilized no-plant conditioned inocula), conditioning soil type and background soil type on the functional groups of fungal OTUs. F value from linear mixed model are presented, *,**,*** indicates significant difference at $P < 0.05$, 0.01 and 0.001, respectively. T value from a *post hoc* test for the pairwise comparison between soil types are also presented. "D,D" indicates conditioned greenhouse soil with live background soil. "F,D" indicates conditioned field soil with live background soil. "D,ST" indicates conditioned greenhouse soil with sterilized background soil. "F.ST" indicates conditioned filed soil with sterilized background soil. Contrasts following a non-significant conditioning soil type and background soil type interaction were not calculated.

	df	Plant pathogen	Saprotroph	Plant symbiont	Endophyte	Unknown	Other
Inocula	10,78	0.92	0.78	1.17	0.74	1.91	1.37
Consoil	1,78	16.74***	4.45*	3.67	1.39	9.79*	10.85*
Backsoil	1,61	0.32	9.76**	5.26*	1.52	3.31	8.98*
Consoil × Backsoil	1,61	22.78***	18.75***	11.67**	0.28	5.12*	4.12*
D,D - F,D		-0.04	0.97	0.50		-0.88	0.88
D,D - D,ST		-2.73*	0.67	0.47		2.74*	-3.67**
D,D - F,ST		3.96**	-4.36***	-2.95*		-1.36	0.42
F,D - D,ST		-2.66*	-0.33	-0.09		3.56**	-4.21***
F,D - F,ST		3.97***	-5.28***	-3.71**		-0.58	-0.38
D,ST - F,ST		6.35***	-4.93***	-3.33**		-3.73**	3.38**
Inocula × Consoil	10,78	1.52	1.47	1.34	0.68	1.65	1.06
Inocula × Backsoil	10,61	1.14	0.78	1.68	0.71	1.77	1.20
Inocula × Consoil × Backsoil	10,61	0.38	1.06	1.74	0.69	0.43	0.72

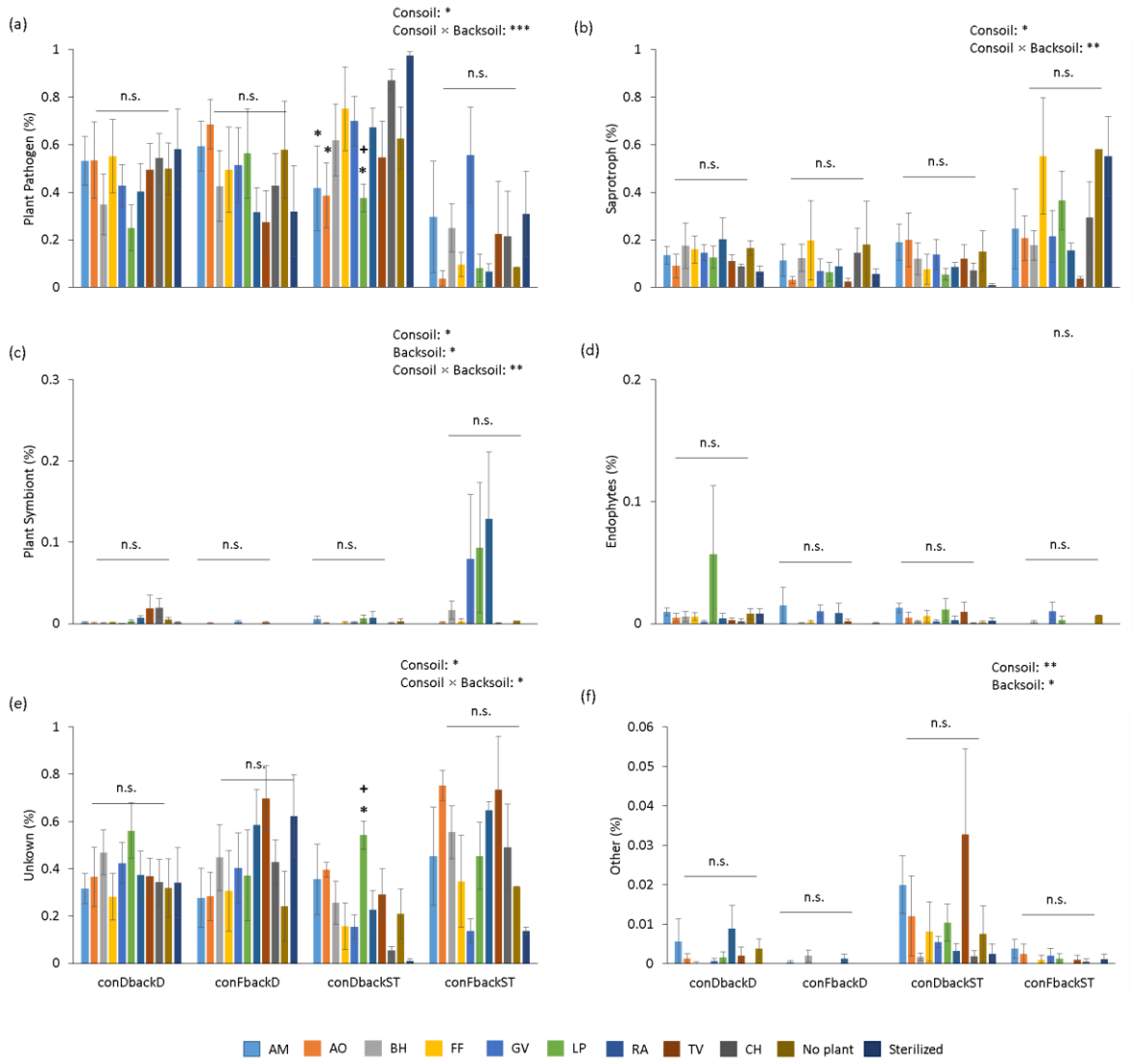

Fig.5.7 The relative abundance of plant pathogenic fungi (a), saprotophic fungi (b), plant symbiontic fungi (c), endophytic fungi (d), fungi with unkown functions (e) and other functional group fungi (d) in different soil treatments. Other functional groups include fungi that are marine species, nematode pathogens, parasite of lichen, fungal parasites and animal pathogens. The overall effects of conditioning plant species, conditioning soil type and background soil type on each fungal functional group were examined, only significant effects are presented in each figure. "*" indicates significant difference compared with sterilized no-plant inoculum in that conditioning soil and background soil combination, "+" indicates significant difference compared with chrysanthemum conspecific inoculum in the soil combination. "n.s." indicates no significant differences between conditioning treatment in that soil type. "conDbackD" indicates conditioned greenhouse soil with live background soil; "conFbackD" indicates conditioned field soil with live background soil; "conDbackST" indicates conditioned greenhouse soil with sterilized background soil; "conFbackST" indicates conditioned field soil with sterilized background soil. Abbreviations of plant species are described in material and methods part, "No plant" in the legend indicates no-plant conditioned inoculum, "Sterilized" in the legend indicates sterilized no-plant soil inoculum.

Table 5.5 The effects of conditioning (all soil treatments, including no-plant soil inocula and sterilized no-plant soil inocula), conditioning soil type and background soil type on OTUs that were highly related with chrysanthemum biomass, and with an average relative abundance were more than 1%. F values following linear mixed model are presented. T values from *post hoc* test for the pairwise comparisons between soil types are also presented. "D,D" indicates conditioned greenhouse soil with live background soil. "F,D" indicates conditioned field soil with live background soil. "D,ST" indicates conditioned greenhouse soil with sterilized background soil. "F.ST" indicates conditioned filed soil with sterilized background soil. *,**,*** indicate significant differences at P<0.05, 0.01 and 0.001, respectively.

	Inocula	Consoil	Backsoil	Consoil × Backsoil							Inocula × Consoil	Inocula × Backsoil	Inocula × Consoil × backsoil
				Overall	D,D - F,D	D,D - D,ST	D,D - F,ST	F,D - D,ST	F,D- F,ST	D,ST- F,ST			
Df	10,80	1,80	1,87	1,87							10,80	10,87	10,87
OTU_5	1.35	1.38	206.45***	8.82**	-1.43	8.19***	10.97***	9.52***	12.24***	2.84*	1.67	1.47	1.03
OTU_9	1.57	0.47	82.60***	11.12**	-1.88	4.20***	7.06***	6.04***	8.84***	2.90*	1.44	2.22*	3.94***
OTU_15	1.42	17.92***	163.30***	29.38***	-0.98	5.32***	11.91***	6.22***	12.73***	6.66***	1.78	2.80**	1.16
OTU_23	1.75	23.47***	287.73***	56.12***	-8.73***	6.86***	8.58***	15.54***	17.19***	1.77	1.25	0.92	0.50
OTU_29	0.59	23.14***	39.84***	16.91***	0.43	1.66	7.89***	1.20	7.34***	6.26***	1.15	1.29	1.79
OTU_14	1.92	79.29***	123.53***	71.30***	-0.41	-1.94	-13.82***	-1.43	-13.72***	-12.02***	1.07	1.72	1.03
OTU_10	1.73	2.07	250.88***	5.87*	0.80	-9.71***	-12.04***	-10.23***	-12.84***	-2.54	1.19	1.78	1.38
OTU_13	2.20*	102.48***	69.46***	50.58***	-2.16	-0.90	-13.10***	1.29	-10.75***	-12.28***	0.85	2.83**	1.05

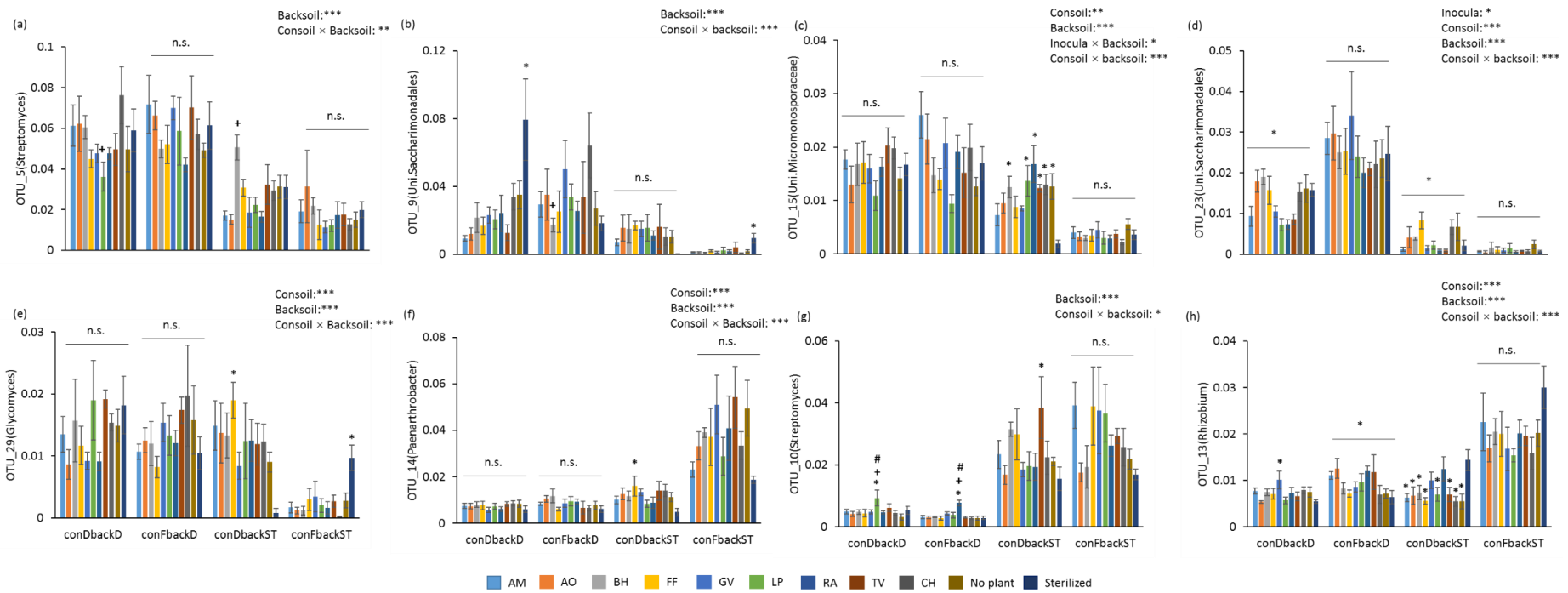

Fig.5.8 The relative abundance of OTUs in different soil treatments. The selection of the eight OTUs is from Table S5.3 that represents OTUs that are highly correlated with plant shoot biomass, and had an average relative abundance across all samples of more than 1%. The correlation between these OTUs and chrysanthemum growth is presented in Fig.S5.3. "*" indicates significant difference compared with sterilized no-plant inoculum in that soil type. If "*" above the bar of sterilized no-plant inoculum, this indicates sterilized no-plant inoculum are significant different from all the other treatments. "+" indicates significant difference compared with chrysanthemum-conditioned inoculum. "#" indicates significant difference compared with no-plant conditioning inoculum. "n.s." indicates no significant differences between conditioning treatments in that soil type. "*" above all bars indicate overall significant effects were found, but no significant differences compared with sterilized no-plant inoculum or no-plant conditioning inoculum or chrysanthemum-conditioned inoculum. Only significant statistics are presented in the upper part of each figure. "conDbackD" indicates conditioned greenhouse soil with live background soil; "conFbackD" indicates conditioned field soil with live background soil; "conDbackST" indicates conditioned greenhouse soil with sterilized background soil; "conFbackST" indicates conditioned field soil with sterilized background soil. "No plant" indicates no-plant conditioning. "Sterilized" indicates sterilized no-plant inocula.

In sterilized background soil, the relative abundance of *Streptomyces* 1 (OTU-5) and Unidentified *Micromonosporaceae* (OTU-15) in the chrysanthemum root microbiome was lower than in live background soil. Addition of conditioned field inocula to sterilized background soil made this pattern stronger than addition of conditioned greenhouse soil inocula to the same background soil (Table 5.5). The relative abundance of *Glycomyces* (OTU-29) decreased, and the relative abundance of *Paenarthrobacter* (OTU-14) and *Rhizobium* (OTU-13) increased in sterilized background soil inoculated with conditioned field soils compared to the other three soil combinations. The relative abundance of *Streptomyces* 2 (OTU-10) in chrysanthemum roots was higher in sterilized than in live background soil (Table 5.5).

Roots of chrysanthemum growing in *Lolium perenne* and *Bromus hordeaceus* soil had lower and higher relative abundances of *Streptomyces* 1 (OTU-5) than roots growing in chrysanthemum conditioned soil, respectively (Fig.5.8a). Roots of chrysanthemum growing in soil with *Festuca filiformis* inoculum had higher relative abundance of *Glycomyces* (OTU-29) and *Paenarthrobacter* (OTU-14) than roots growing with sterilized inocula (Fig.5.8e,f). Inoculation of *Lolium perenne*, *Galium verum* and *Tanacetum vulgare* soil resulted in higher relative abundance of *Streptomyces* 2 (OTU-10) in chrysanthemum roots than inoculation with sterilized soil, chrysanthemum soil, or no-plant conditioned soil (Fig.5.8g). Chrysanthemum grown with 100% sterilized soil had a higher relative abundance of *Rhizobium* (OTU-13) than plants grown with plant conditioned inocula (except *Rumex acetosella* and *Galium verum*) (Fig.5.8h). The differences between the effects of conditioning plant species were all observed in soils that contained either conditioned greenhouse soil or live background soil (Fig.5.8).

Discussion

We show that inoculation of soil microbiomes at the start of a chrysanthemum growth cycle leads to differences in chrysanthemum root microbiomes at the end of this growth cycle and hence that these inoculated microbiomes established in the soil. Remarkably, this was also true in live background soil that contained a microbiome already. However, inoculating conditioned field soil into sterilized background soil was the best soil combination for chrysanthemum performance, and led to the most distinctive structure of chrysanthemum root microbiome. Chrysanthemum growth was negatively influenced in live greenhouse soil and inoculation of field soil or greenhouse soil conditioned by wild plant species into this soil did not significantly improve chrysanthemum growth in these soils. However, in terms of the chrysanthemum root microbiome, inoculation with soil conditioned by wild plant species significantly influenced the bacterial diversity and the relative abundance of OTUs that were both positively and negatively correlated with chrysanthemum growth, and reduced the relative abundance of pathogenic fungi. Chrysanthemum biomass was highest in sterilized soil but also the relative

abundance of plant pathogenic fungi was higher than in inoculated soils. Another important finding is that in this study, plant susceptibility to thrips was not influenced by inoculation, and we did not find any significant correlations between root-associated microbes and thrips performance.

The effects of inoculation on the chrysanthemum root microbiome were more obvious than on shoot biomass of the plant. In terms of root pathogenic fungi and bacterial diversity in chrysanthemum roots, inoculation with soil from wild plant species either showed no significant effects or led to lower relative abundance of pathogenic fungi and higher bacterial diversity both when compared with sterilized inocula or with an inoculum of chrysanthemum soil. Comparing with domesticated crops, plant species that grow in natural soils typically have more diverse rhizosphere microbiomes, which may also increase the microbial diversity in the roots of plants that grow later in these soils (Pérez-Jaramillo et al. 2016; Mariotte et al. 2017). One specific conditioned soil which influenced chrysanthemum root microbiome in a consistent direction, is soil conditioned by *Lolium perenne*, which strongly affected the relative abundance of *Streptomyces*. Other work demonstrated that *Lolium perenne* increases the abundance of soil bacterial groups that have antagonistic activities against soil pathogenic fungi (Latz et al. 2015; 2016). In the current study, these changes induced by *Lolium perenne* conditioning did not significantly influence chrysanthemum biomass. In previous studies using the same system, root biomass was always more responsive to different soil treatments than shoot biomass of chrysanthemum (Ma et al. 2017; 2018). Unfortunately, we were unable to measure root biomass in this study because these samples were used for the molecular analysis of the root microbiome.

It is plausible that plant growth in our study was not solely determined by the increase or decrease in the specific groups of microbes. Because the functional capacity of the plant microbiome is more than the sum of its individual groups and the influence of the root microbiome on plant growth is the net effect of all interactions between the beneficial and detrimental microbes (van der Heijden and Hartmann 2016; Kaplan et al. 2018). For example, inoculation with *Festuca filiformis* conditioned soils led to overall higher bacterial diversity on chrysanthemum roots and also a higher relative abundance of both positive and negative plant growth-correlated OTUs. *Festuca filiformis* was also the only wild plant species that conditioned soil in a way that resulted in higher chrysanthemum biomass after inoculation than inoculation with chrysanthemum conditioned soil, indicating that the net effects of the community may be more important than the changes in the specific groups. The changes in chrysanthemum root microbiomes induced by inoculation of soils conditioned by wild plant species could also be functional redundant, and therefore did not lead to the changes in the overall influence the root microbiome on chrysanthemum biomass (Allison and Martiny 2008). Hence, our results emphasize that metagenomics sequencing, which is commonly used nowadays, can be an important tool in examining plant-soil

feedbacks, and soils inoculations (Nesme et al. 2016), but that this method may not be sufficient to disentangle the causal effects and mechanisms.

Our results also highlight that the benefit of sterilizing soil in this cultivation is short-term. In the short-term, *i.e.* the first growth cycle after sterilization, sterilized soil provides the best chrysanthemum yield (Mahmood et al. 2014; Gebhardt et al. 2017). However, at the same time, soil sterilization can negatively influence the soil biota that could suppress infections of soil-borne diseases to the plant. For example, soil sterilization can reduce the spore attachment of a beneficial bacteria to the plant parasitic nematode *Meloidogyne arenaria* (Liu et al. 2017). In the current study, we observed two potential negative effects of sterilized soil on chrysanthemum. First, sterilized soil enriched the colonization of root-associated pathogenic fungi in plant roots compared with inoculated soils. Second, when inoculating conditioned greenhouse soil inocula which were bad for chrysanthemum growth and may potentially contain higher abundance of pathogens into sterilized background soil, the relative abundance of pathogenic fungi on chrysanthemum was even higher than after inoculating the same inocula into live greenhouse background soil. The dominant pathogenic fungi in this study was *Olpidium brassicae.* Apart from being a pathogen, *Olpidium* can be a transmission vector of viruses to host plant species by creating wounds in the host (Campbell 1996; Raaijmakers et al. 2009). Thus, because of these negative effects of soil sterilization on the soil microbial community, the yield of chrysanthemum in sterilized soil is likely to decline in the longer-term. Indeed, in a previous study, we observed that in the second growth cycle, chrysanthemum growth in originally sterilized soil decreased sharply, and that inoculation of plant-conditioned soils at the start of the first growth cycle reduced such negative effects (Ma et al. 2018). Thus, negative effects of soil sterilization on soil microbial communities are likely to cause negative effects on plant growth in the longer term in chrysanthemum.

The relative abundance of some bacterial phyla, such as *Chloroflexi, Verrucomicrobia, Armatimonadetes*, were highest in the best soil combination for chrysanthemum growth, and were lowest in the worst soil combination for chrysanthemum growth, indicating these bacterial phyla were associated with chrysanthemum growth. *Chloroflexi* and *Verrucomicrobia* were reported in previous studies as being enriched in disease suppressive soils against fungal pathogens (Xiong et al. 2017; Sanguin et al. 2009). *Patescibacteria* responded to the conditioning soil type and background soil type in the opposite direction, and thus may be negatively associated with plant biomass. *Patescibacteria* is a phylum with a presumed plant symbiotic or parasitic lifestyle (Sánchez-Osuna et al. 2017). It is possible that microbes with this lifestyle are costly for chrysanthemum and hence reduce growth. Moreover, chrysanthemum is known to form associations with arbuscular mycorrhizal fungi (del Mar Montiel-Rozas et al. 2016; Sohn et al. 2003; D'Amelio et al. 2011), but in this study, no mycorrhizal

fungi was detected in the roots even though the primers amplify also AMF. It is possible that with the high nutrient supply that we used following the recommendation of growth advisors, chrysanthemum plants do not need to form symbiosis with AMF.

Among the eight most abundant chrysanthemum growth-correlated OTUs, there were two *Streptomyces* spp, indicating a potentially important role of *Streptomyces* spp for chrysanthemum growth. *Streptomyces* spp are known for their capabilities to compete for plant-produced resources including root exudates and dead plant tissue, often form an intimate association with plants and are common colonists of the rhizosphere and endosphere (Cao et al. 2004; Viaene et al. 2016; Franco et al. 2016; Schlatter et al. 2017). The mechanisms of beneficial *Streptomyces* strains that promote plant growth involve auxin production, production of antibiotics against plant pathogens, inducing systematic resistance of plants against the attack by pathogens and emission of volatile organic compounds that stimulate plant growth (Viaene et al. 2016). Manipulative studies have found that inoculation of beneficial *Streptomyces* strains resulted in an increase in plant biomass in crops such as rice, wheat, sorghum and tomato (Gopalakrishnan et al. 2013; 2014; Jog et al. 2014; Palaniyandi et al. 2014). Our study also provides evidence that this specific *Streptomyces* strain (OTU-10) not only had a high relative abundance in the root microbiome but also positively correlated with the growth of chrysanthemum crop. The *Streptomyces* genus also contains species with phytopathogenic features, such as the potato scab disease caused by *Streptomyces scabies* (Weller et al. 2002). In our study, one *Streptomyces* strain (OTU-5) with high relative abundance correlated negatively with chrysanthemum growth. It is important to note that correlations between microbial OTUs that are associated to the shoot biomass do not provide information about the causal relationships between these two. It is possible, for example, that increased growth of the plant stimulates or reduces the density of specific OTUs via changes in root exudation patterns rather than that these specific OTUs stimulate or reduce the growth of the plant. Manipulative studies are needed in the future to reveal the causal effects between these important OTUs and chrysanthemum performance.

The changes in root microbiome or in shoot biomass of chrysanthemum did not significantly influence the performance of thrips. This is in contrast with previous studies that found changes in the composition or function of root-associated microbes can reduce or increase the aboveground defense of plants (Badri et al. 2013; Pieterse et al. 2014; Kos et al. 2015). The difference between their study and this study is the performance of thrips in this study was tested on a detached leaf taken from the plant. Hence, the response of chrysanthemum to thrips, such as the induced systematic resistance by beneficial microbes, was not measured. Effectively, in our study we tested whether changes in the leaf defense compounds of chrysanthemum due to growing in different soils influenced the performances of thrips (Wang et al.

2015). In a previous study, we found that the concentration of chlorogenic acid, which has been reported to be an important plant defense compound against thrips in chrysanthemum leaves (Leiss et al. 2009), was positively correlated with chrysanthemum shoot biomass (Ma et al. 2017). However, in the current study, the increase in chrysanthemum shoot biomass was not related to the performance of thrips and we did not measure chlorogenic acid. Remarkably, a meta-analysis about the influences of plant traits and secondary metabolites on plant resistance to herbivores found that there was no overall association between the concentrations of defense compounds with the herbivore susceptibility (Carmona et al. 2011). Further studies are need to analyse the leaf metabolome of chrysanthemum growing in different soils, to infer whether these metabolomes change depending on the soil inoculation used and how this relates to the performance of thrips.

In conclusion, this study highlights the potential of using soil from natural ecosystems to improve chrysanthemum performance in commercial greenhouses. Soil inoculation in greenhouse soil did not cause significant effects on chrysanthemum growth but altered the chrysanthemum root microbiome. Plant species such as *Lolium perenne*, *Festuca filiformis*, changed the soil so that inoculation with this soil increased the bacterial diversity and the abundance of positive and negative plant growth-correlated OTUs, and reduced the relative abundance of pathogenic fungi in the root-associated microbiome of chrysanthemum. Chrysanthemum biomass was highest in sterilized soil, but in this soil the root pathogen load was also highest, potentially leading to pathogen outbreak and hence sterilization without inoculation may not be a sustainable strategy. The root-associated fungal communities in chrysanthemum growing in live greenhouse soil were dominated by pathogenic fungi phylum *Olpidiomycota*. The bacteria phyla *Patescibacteria*, *Chloroflexi*, *Verrucomicrobia*, *Armatimonadetes* were related most strongly to changes in plant growth. Among the eight OTUs that were abundant and that highly correlated with plant growth, two of them were from *Streptomyces* spp. Future studies should explore the causal relationships between these strains and chrysanthemum growth.

Supplementary material

Standardization of sequencing data

For bacterial data, the total number of reads per sample were ranged from 1467 to 85096, samples with total number of reads less than 8000 were removed. There were 9 samples removed, they are AO2FD, AO4DD, AM2FD, FF5FST, LP2FST, TV4DST, TV5FD, TV3FD, ST5DD. Then, OTUs with total number of reads less than 3 were also removed. For each sample, abundance of each OTU was transformed by dividing it by the total amount of reads per sample (McMurdie and Holmes 2014). Further, OTUs with abundance less than 0.000125 were removed. The relationships between total number of reads with total number of OTUs before and after the standardization are shown in Fig.S5.1 (a,b). For fungal data, the sequencing of sample "TV3FD" failed. Therefore, in total, there were 219 samples. The total number of fungal reads per sample range from 1 to 9701 as plant material from chrysanthemum roots was co-amplified. Samples with less than 140 reads were removed. There were 93 samples were removed. OTUs with less than 3 reads were then removed. For each sample, abundance of each OTU was transformed by dividing it by the total amount of reads per sample (McMurdie and Holmes 2014). OTUs with abundance less than 0.0069 were removed. The relationships between total number of reads with total number of OTUs before and after the standardization are shown in Fig.S5.1 (c,d). The transformed abundance data were used for all analysis of the root microbiome.

Table S5.1 The effects of conditioning plant species (all soil treatments), conditioning soil type and background soil type on the bacterial phyla composition. F-values following linear mixed model are presented. T-values from *post hoc* test for the pairwise comparisons between soil types are presented. "D,D" indicates conditioned disease soil with background disease soil. "D,ST" indicates conditioned disease soil with sterilized background soil. "F,D" indicates conditioned field soil with disease background soil. "F.ST" indicates conditioned filed soil with sterilized background soil. *,**,*** indicate significant differences at P<0.05, 0.01 and 0.001, respectively.

Bacterial phylum	Inocula	Consoil	Backsoil	Consoil × Backsoil						Inocula × Consoil	Inocula × Backsoil	Inocula × Consoil × backsoil
				Overall	D,D-F,D	D,D- D,ST	D,D - F,ST	F,D - D,ST	F,D - F,ST	D,ST-F,ST			
df	10,80	1,80	1,87	1,87							10,80	10,87	10,87
Proteobacteria	1.31	5.64*	1.18	0.96							1.78	0.60	0.63
Actinobacteria	5.25***	0.34	9.53**	5.79*	1.40	-0.59	-2.58	-1.99	-3.94***	-2.01	1.70	1.72	0.61
Patescibacteria	0.84	3.19	180.72***	4.82*	-0.31	8.04***	10.75***	8.23***	10.91***	2.77*	0.64	0.47	1.36
Bacteroidetes	1.82	8.62*	40.61***	20.98***	1.08	-1.16	-6.17***	-2.13	-7.86***	-5.15***	1.41	1.73	0.47
Cyanobacteria	2.09*	0.70	1.26	2.02							1.25	1.07	1.07
Planctomycetes	2.10*	1.08	2.61	0.41							0.83	0.82	0.45
Chloroflexi	1.22	9.93**	127.83***	9.59**	0.06	-5.86***	-10.23***	-5.85***	-10.15***	-4.42***	4.19***	0.58	3.65**
Acidobacteria	1.70	19.45***	10.12**	9.18**	0.95	0.13	5.32***	-0.82	4.29***	5.21***	0.93	0.63	0.15
Verrucomicrobia	1.72	10.29**	123.71***	13.39**	0.25	-5.35***	-10.24***	-5.53***	-10.35***	-4.95***	2.05*	1.71	2.09*
Firmicutes	0.36	14.08***	3.01	5.58*	-1.35	0.37	-3.84***	1.68	-2.80	-4.20***	0.38	0.80	0.49
Gemmatimonadetes	0.51	0.42	2.20	1.79							1.13	0.47	1.75
Armatimonadetes	3.01**	23.06***	203.39***	45.01***	1.32	-5.48***	-13.76***	-6.73***	-14.89***	-8.35***	2.15*	2.57**	2.75**
Chlamydiae	1.09	0.27	4.27*	1.99							0.87	0.93	0.77
Dependentiae	2.27*	9.43**	27.64***	0.28							0.94	2.47**	2.73**
low.abundance	4.38***	1.70	3.91*	3.57							1.32	1.44	1.06

Table S5.2 The effects of conditioning plant species (all soil treatments), conditioning soil type and background soil type on the fungal phyla composition. F-values following linear mixed model are presented. T-values from *post hoc* test for the pairwise comparisons between soil types are presented. "D,D" indicates conditioned disease soil with background disease soil. "D,ST" indicates conditioned disease soil with sterilized background soil. "F,D" indicates conditioned field soil with disease background soil. "F.ST" indicates conditioned filed soil with sterilized background soil. *,**,*** indicate significant differences at P<0.05, 0.01 and 0.001, respectively.

Fungal phylum	Inocula	Consoil	Backsoil	Consoil × Backsoil						Inocula × Consoil	Inocula × Backsoil	Inocula × Consoil × backsoil
				Overall	D,D-F,D	D,D-D,ST	D,D - F,ST	F,D - D,ST	F,D-F,ST	D,ST-F,ST			
df	10,78	1,78	1,61	1,61							10,78	10,61	10,61
Olpidiomycota	0.88	14.60**	0.15	23.33***	-0.30	-2.91*	3.64**	-2.57	3.88**	6.19***	1.53	1.20	0.31
Ascomycota	2.06*	20.11***	8.36**	49.78***	1.18	2.47	-6.05***	1.26	-7.04***	-8.22***	1.19	0.95	0.63
Basidiomycota	1.07	0.00	0.14	0.13							1.16	0.68	1.13
Mortierellomycota	4.46***	2.75	9.26**	4.14*	0.14	-0.39	-3.34**	-0.53	-3.44**	-3.00*	3.61**	2.39*	3.79**
Rozellomycota	2.21*	4.51*	0.63	2.04							0.44	0.53	1.37
Chytridiomycota	0.54	6.10*	5.13*	0.57							0.29	0.97	0.94
Entomophthoromycota	0.81	1.07	1.56	1.11							0.61	0.97	0.65
Glomeromycota	0.83	1.62	1.55	2.28							0.88	0.71	0.90
Mucoromycota	0.89	9.43*	11.79*	16.91***	0.04	0.00	-5.48***	-0.04	-5.69***	-5.48***	1.15	1.51	2.54*
unidentified	0.47	0.03	14.41**	2.71							0.36	1.65	0.37

Fig.S5.1 Relationships between total number of OTUs with total number of reads per sample. Panel a and b show bacterial OTUs and reads before and after standardization, respectively. Panel c and d show fungal OTUs and reads before and after standardization, respectively.

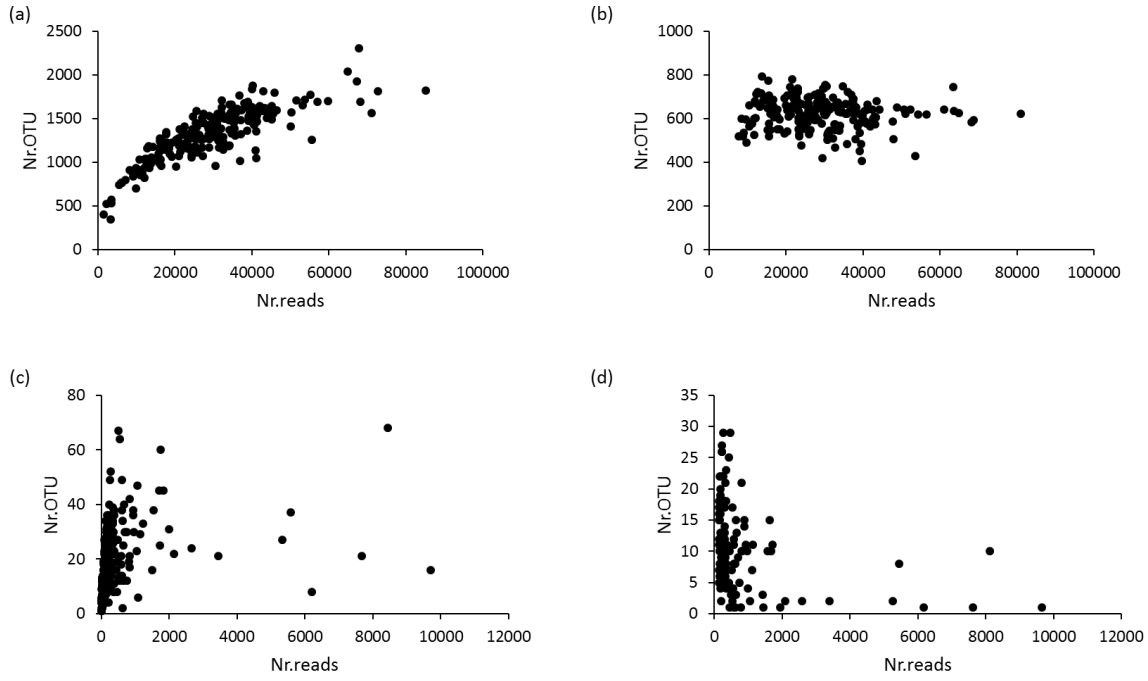

Fig.S5.2 Correlations between bacterial diversity and fungal diversity to body length of female and male thrips. "n.s." indicates no significant correlation was found using Pearson correlation.

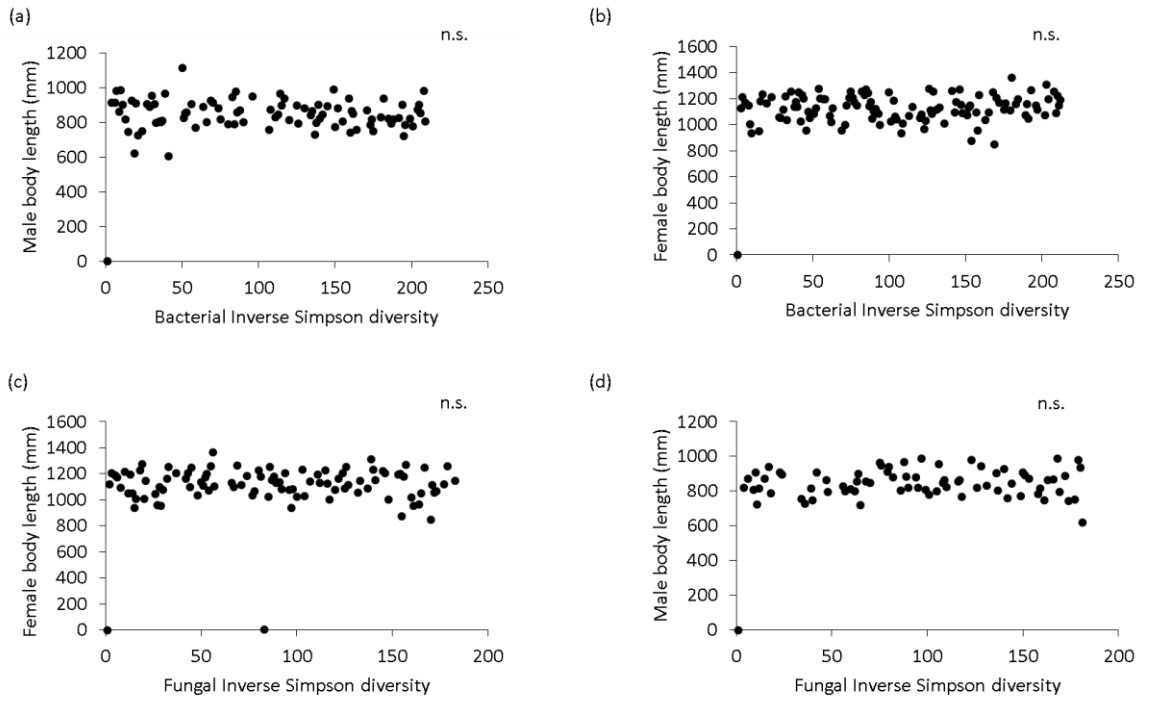

Fig.S5.3 OTUs which were highly related with chrysanthemum shoot biomass and with an average relative abundance over 1%. R and *P*-values following Pearson correlations are presented on each figure.

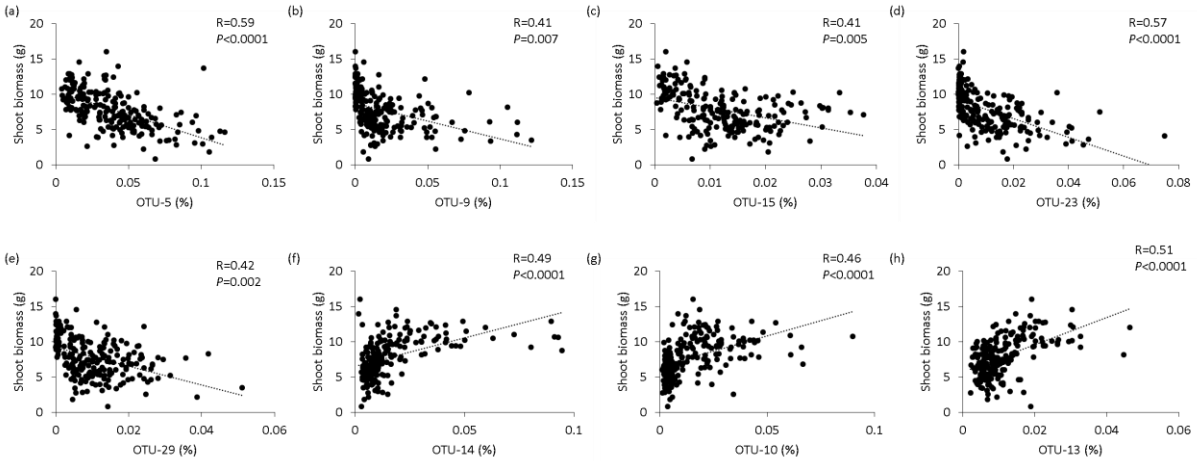

Table S5.3 Chrysanthemum growth-correlated OTUs. R following a Pearson correlation is presented for each OTU, the positive and negative of R indicate the positive and negative correlation between OTU and chrysanthemum biomass, respectively. "Uni" in the genus name indicates unidentified.

OTUs	Phylum	Genus	R
OTU_652	Acidobacteria	Blastocatella	-0.47634
OTU_903	Acidobacteria	Bryobacter	0.439812
OTU_647	Acidobacteria	Bryobacter	0.489648
OTU_597	Acidobacteria	Subgroup_10	-0.54509
OTU_585	Acidobacteria	Subgroup_10	-0.48128
OTU_883	Acidobacteria	Uni.Acidobacteria	-0.44697
OTU_1417	Acidobacteria	Uni.Acidobacteria	-0.38933
OTU_187	Acidobacteria	Uni.Blastocatellaceae	0.422538
OTU_609	Acidobacteria	Uni.Blastocatellia_(Subgroup_4)	-0.49561
OTU_33	Actinobacteria	Aeromicrobium	0.466094
OTU_752	Actinobacteria	Agromyces	-0.48071
OTU_1047	Actinobacteria	Angustibacter	0.451613
OTU_277	Actinobacteria	Cellulosimicrobium	-0.49631
OTU_1873	Actinobacteria	CL500-29_marine_group	0.441974
OTU_1372	Actinobacteria	Demequina	-0.39926
OTU_1726	Actinobacteria	Fodinicola	0.407739
OTU_879	Actinobacteria	Geodermatophilus	0.477513
OTU_29	Actinobacteria	Glycomyces	-0.42213
OTU_1477	Actinobacteria	Haloactinopolyspora	0.560683
OTU_1750	Actinobacteria	Iamia	0.399671
OTU_907	Actinobacteria	Iamia	0.418951
OTU_1031	Actinobacteria	Iamia	0.431053
OTU_328	Actinobacteria	Iamia	0.444016
OTU_1196	Actinobacteria	Iamia	0.461396
OTU_259	Actinobacteria	Iamia	0.462532
OTU_423	Actinobacteria	Iamia	0.614482
OTU_808	Actinobacteria	Ilumatobacter	-0.47066
OTU_159	Actinobacteria	Marmoricola	0.577897
OTU_456	Actinobacteria	Microbacterium	0.567982
OTU_713	Actinobacteria	Mycobacterium	0.397728
OTU_228	Actinobacteria	Mycobacterium	0.486204
OTU_453	Actinobacteria	Nocardioides	-0.44616
OTU_247	Actinobacteria	Nocardioides	0.392475
OTU_770	Actinobacteria	Nocardioides	0.39304
OTU_399	Actinobacteria	Nocardioides	0.400249
OTU_325	Actinobacteria	Nocardioides	0.421491
OTU_413	Actinobacteria	Nocardioides	0.426096
OTU_1080	Actinobacteria	Nocardioides	0.430078
OTU_779	Actinobacteria	Nocardioides	0.489582
OTU_575	Actinobacteria	Nocardioides	0.533037
OTU_88	Actinobacteria	Nocardioides	0.533118
OTU_5643	Actinobacteria	Nocardioides	0.544101

OTUs	Phylum	Genus	R
OTU_185	Actinobacteria	Nocardioides	0.631145
OTU_4057	Actinobacteria	Paenarthrobacter	0.435646
OTU_14	Actinobacteria	Paenarthrobacter	0.489107
OTU_127	Actinobacteria	Phycicoccus	0.516922
OTU_610	Actinobacteria	Pseudonocardia	0.403993
OTU_912	Actinobacteria	Rhodococcus	0.468061
OTU_576	Actinobacteria	Streptomyces	-0.60253
OTU_5	Actinobacteria	Streptomyces	-0.58886
OTU_580	Actinobacteria	Streptomyces	-0.53758
OTU_1960	Actinobacteria	Streptomyces	-0.45851
OTU_297	Actinobacteria	Streptomyces	-0.45337
OTU_1775	Actinobacteria	Streptomyces	-0.4529
OTU_2360	Actinobacteria	Streptomyces	-0.43477
OTU_3833	Actinobacteria	Streptomyces	0.403153
OTU_2714	Actinobacteria	Streptomyces	0.412275
OTU_2027	Actinobacteria	Streptomyces	0.417039
OTU_10	Actinobacteria	Streptomyces	0.462477
OTU_169	Actinobacteria	Streptomyces	0.483204
OTU_1677	Actinobacteria	Streptomyces	0.485293
OTU_279	Actinobacteria	Streptomyces	0.501712
OTU_44	Actinobacteria	Streptomyces	0.634779
OTU_623	Actinobacteria	Terrabacter	0.470358
OTU_1048	Actinobacteria	Uni.Acidimicrobiia	0.445889
OTU_669	Actinobacteria	Uni.Actinomarinales	-0.4734
OTU_154	Actinobacteria	Uni.Intrasporangiaceae	0.407171
OTU_434	Actinobacteria	Uni.Micrococcaceae	0.548598
OTU_50	Actinobacteria	Uni.Micrococcaceae	0.555612
OTU_15	Actinobacteria	Uni.Micromonosporaceae	-0.4123
OTU_420	Actinobacteria	Uni.Micromonosporaceae	-0.40419
OTU_335	Actinobacteria	Uni.Microtrichales	0.445566
OTU_548	Actinobacteria	Uni.Nocardioidaceae	0.444814
OTU_165	Actinobacteria	Uni.Solirubrobacterales	-0.61799
OTU_108	Actinobacteria	Uni.Solirubrobacterales	-0.60189
OTU_104	Actinobacteria	Uni.Solirubrobacterales	-0.58795
OTU_200	Actinobacteria	Uni.Solirubrobacterales	-0.43583
OTU_661	Actinobacteria	Uni.Streptomycetaceae	0.418161
OTU_895	Armatimonadetes	Uni.Armatimonadales	0.431331
OTU_1823	Armatimonadetes	Uni.Armatimonadetes	0.405588
OTU_1326	Armatimonadetes	Uni.Armatimonadetes	0.491623
OTU_440	Armatimonadetes	Uni.Fimbriimonadaceae	0.417749
OTU_442	Armatimonadetes	Uni.Fimbriimonadaceae	0.433463
OTU_208	Bacteroidetes	Chitinophaga	0.421041
OTU_305	Bacteroidetes	Chryseolinea	-0.58237
OTU_701	Bacteroidetes	Chryseolinea	-0.46925
OTU_1531	Bacteroidetes	Chryseolinea	-0.42683

OTUs	Phylum	Genus	R
OTU_1120	Bacteroidetes	Chryseolinea	0.397513
OTU_1829	Bacteroidetes	Chryseolinea	0.418744
OTU_319	Bacteroidetes	Chryseolinea	0.462366
OTU_173	Bacteroidetes	Emticicia	0.4921
OTU_717	Bacteroidetes	Flavisolibacter	0.413845
OTU_850	Bacteroidetes	Flavisolibacter	0.419859
OTU_1019	Bacteroidetes	Flavisolibacter	0.437309
OTU_1254	Bacteroidetes	Flavisolibacter	0.52846
OTU_391	Bacteroidetes	Flavitalea	0.507189
OTU_1352	Bacteroidetes	Flavitalea	0.520299
OTU_497	Bacteroidetes	Flavitalea	0.550851
OTU_675	Bacteroidetes	Fluviicola	0.427183
OTU_438	Bacteroidetes	Lacibacter	0.393284
OTU_2270	Bacteroidetes	Larkinella	0.464173
OTU_217	Bacteroidetes	Niastella	-0.58619
OTU_77	Bacteroidetes	Niastella	0.482436
OTU_602	Bacteroidetes	Pedobacter	0.3912
OTU_2757	Bacteroidetes	Pedobacter	0.418965
OTU_1622	Bacteroidetes	Pedobacter	0.420375
OTU_109	Bacteroidetes	Pedobacter	0.54717
OTU_2246	Bacteroidetes	Pseudoflavitalea	0.443741
OTU_1054	Bacteroidetes	Sporocytophaga	-0.43169
OTU_536	Bacteroidetes	Terrimonas	0.392563
OTU_1932	Bacteroidetes	Uni.Chitinophagaceae	-0.43706
OTU_1276	Bacteroidetes	Uni.Chitinophagaceae	0.443779
OTU_714	Bacteroidetes	Uni.Chitinophagaceae	0.492224
OTU_562	Bacteroidetes	Uni.Chitinophagaceae	0.504909
OTU_667	Bacteroidetes	Uni.Ignavibacteria	-0.50274
OTU_58	Bacteroidetes	Uni.Microscillaceae	-0.66381
OTU_564	Bacteroidetes	Uni.Microscillaceae	-0.60225
OTU_533	Bacteroidetes	Uni.Microscillaceae	-0.59843
OTU_301	Bacteroidetes	Uni.Microscillaceae	-0.57591
OTU_586	Bacteroidetes	Uni.Microscillaceae	-0.53081
OTU_311	Bacteroidetes	Uni.Microscillaceae	-0.51004
OTU_196	Bacteroidetes	Uni.Microscillaceae	-0.47692
OTU_1110	Bacteroidetes	Uni.Microscillaceae	-0.45232
OTU_351	Bacteroidetes	Uni.Microscillaceae	0.413534
OTU_121	Bacteroidetes	Uni.Microscillaceae	0.415411
OTU_614	Bacteroidetes	Uni.Microscillaceae	0.420309
OTU_1006	Bacteroidetes	Uni.Microscillaceae	0.437237
OTU_989	Bacteroidetes	Uni.Rhodothermaceae	0.409612
OTU_289	Bacteroidetes	Uni.Sphingobacteriaceae	0.411957
OTU_5349	Chlamydiae	Uni.Chlamydiales	0.423321
OTU_1018	Chloroflexi	FFCH7168	0.427277
OTU_166	Chloroflexi	FFCH7168	0.496753

OTUs	Phylum	Genus	R
OTU_333	Chloroflexi	FFCH7168	0.522302
OTU_1140	Chloroflexi	Uni.Anaerolineae	-0.41436
OTU_1331	Chloroflexi	Uni.Anaerolineae	0.424423
OTU_106	Chloroflexi	Uni.Ardenticatenaceae	0.570648
OTU_1101	Chloroflexi	Uni.Ardenticatenales	-0.42171
OTU_709	Chloroflexi	Uni.Ardenticatenales	-0.3973
OTU_759	Chloroflexi	Uni.Caldilineaceae	0.398028
OTU_643	Chloroflexi	Uni.Chloroflexi	-0.49307
OTU_605	Chloroflexi	Uni.Chloroflexi	-0.44349
OTU_5702	Chloroflexi	Uni.Chloroflexi	-0.39581
OTU_1099	Chloroflexi	Uni.Kallotenuales	0.412174
OTU_1143	Chloroflexi	Uni.Kallotenuales	0.457317
OTU_182	Chloroflexi	Uni.Roseiflexaceae	-0.59452
OTU_891	Chloroflexi	Uni.Roseiflexaceae	0.446741
OTU_1380	Chloroflexi	Uni.Roseiflexaceae	0.476749
OTU_212	Chloroflexi	Uni.Roseiflexaceae	0.530794
OTU_601	Chloroflexi	Uni.Roseiflexaceae	0.532884
OTU_47	Chloroflexi	Uni.Roseiflexaceae	0.59337
OTU_572	Chloroflexi	Uni.SBR1031	-0.48816
OTU_507	Chloroflexi	Uni.SBR1031	-0.41624
OTU_2009	Chloroflexi	Uni.SBR1031	-0.41046
OTU_2070	Chloroflexi	Uni.SBR1031	0.432582
OTU_1439	Chloroflexi	Uni.Thermomicrobiales	0.413626
OTU_1723	Chloroflexi	Uni.Thermomicrobiales	0.466882
OTU_991	Cyanobacteria	Uni.Sericytochromatia	0.410722
OTU_425	Cyanobacteria	Uni.Sericytochromatia	0.550732
OTU_429	Cyanobacteria	Uni.Sericytochromatia	0.619879
OTU_518	Firmicutes	Paenibacillus	-0.45929
OTU_1597	Gemmatimonadetes	Gemmatimonas	0.432686
OTU_392	Gemmatimonadetes	Uni.Gemmatimonadaceae	-0.48351
OTU_1498	Gemmatimonadetes	Uni.Gemmatimonadaceae	-0.39299
OTU_818	Gemmatimonadetes	Uni.Gemmatimonadaceae	0.398241
OTU_1385	Gemmatimonadetes	Uni.Gemmatimonadaceae	0.478934
OTU_227	Patescibacteria	Uni.Saccharimonadaceae	0.505487
OTU_23	Patescibacteria	Uni.Saccharimonadales	-0.56967
OTU_164	Patescibacteria	Uni.Saccharimonadales	-0.4772
OTU_270	Patescibacteria	Uni.Saccharimonadales	-0.47094
OTU_9	Patescibacteria	Uni.Saccharimonadales	-0.4092
OTU_771	Patescibacteria	Uni.Saccharimonadales	-0.39196
OTU_599	Patescibacteria	Uni.Saccharimonadales	0.392211
OTU_1436	Patescibacteria	Uni.Saccharimonadales	0.39298
OTU_718	Patescibacteria	Uni.Saccharimonadales	0.402183
OTU_346	Patescibacteria	Uni.Saccharimonadales	0.430612
OTU_1499	Planctomycetes	Fimbriiglobus	-0.41838
OTU_1760	Planctomycetes	Gemmata	0.399695

OTUs	Phylum	Genus	R
OTU_408	Planctomycetes	Gemmata	0.410851
OTU_1030	Planctomycetes	Gemmata	0.475908
OTU_99	Planctomycetes	Pir4_lineage	-0.71645
OTU_338	Planctomycetes	Pir4_lineage	-0.6647
OTU_517	Planctomycetes	Pir4_lineage	-0.61581
OTU_1327	Planctomycetes	Pir4_lineage	-0.61278
OTU_436	Planctomycetes	Pir4_lineage	-0.60434
OTU_229	Planctomycetes	Pir4_lineage	-0.57211
OTU_922	Planctomycetes	Pir4_lineage	-0.55433
OTU_825	Planctomycetes	Pir4_lineage	-0.51597
OTU_810	Planctomycetes	Pir4_lineage	-0.49145
OTU_832	Planctomycetes	Pir4_lineage	-0.48392
OTU_846	Planctomycetes	Pir4_lineage	-0.47857
OTU_722	Planctomycetes	Pir4_lineage	-0.42856
OTU_927	Planctomycetes	Pir4_lineage	-0.41876
OTU_811	Planctomycetes	Pirellula	-0.45705
OTU_876	Planctomycetes	Pirellula	0.426637
OTU_143	Planctomycetes	Pirellula	0.469724
OTU_1261	Planctomycetes	Pirellula	0.476333
OTU_367	Planctomycetes	Planctomicrobium	-0.44359
OTU_1646	Planctomycetes	Planctomicrobium	-0.43912
OTU_330	Planctomycetes	Rhodopirellula	-0.55899
OTU_645	Planctomycetes	Rhodopirellula	0.458468
OTU_370	Planctomycetes	SH-PL14	-0.65176
OTU_748	Planctomycetes	SH-PL14	-0.48059
OTU_618	Planctomycetes	SH-PL14	-0.42362
OTU_685	Planctomycetes	SH-PL14	-0.41311
OTU_820	Planctomycetes	SH-PL14	0.434482
OTU_829	Planctomycetes	SH-PL14	0.439917
OTU_243	Planctomycetes	SH-PL14	0.462753
OTU_300	Planctomycetes	SH-PL14	0.486571
OTU_1636	Planctomycetes	SH-PL14	0.497136
OTU_2368	Planctomycetes	Singulisphaera	0.417316
OTU_1599	Planctomycetes	Uni.Isosphaeraceae	0.416532
OTU_1700	Planctomycetes	Uni.Isosphaeraceae	0.449928
OTU_776	Planctomycetes	Uni.Isosphaeraceae	0.496409
OTU_998	Planctomycetes	Uni.Pirellulaceae	-0.44482
OTU_707	Planctomycetes	Uni.Planctomycetales	-0.46694
OTU_1194	Planctomycetes	Uni.Planctomycetales	-0.46594
OTU_753	Planctomycetes	Uni.Planctomycetales	-0.39279
OTU_1161	Planctomycetes	Uni.Planctomycetales	0.456942
OTU_995	Planctomycetes	Uni.Planctomycetales	0.46452
OTU_1770	Planctomycetes	Uni.Tepidisphaerales	0.391199
OTU_1210	Planctomycetes	Uni.Tepidisphaerales	0.448376
OTU_110	Proteobacteria	[Rhizobium]_sphaerophysae_group	-0.39068

OTUs	Phylum	Genus	R
OTU_581	Proteobacteria	[Rhizobium]_sphaerophysae_group	-0.38975
OTU_189	Proteobacteria	Acidibacter	0.406473
OTU_275	Proteobacteria	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.435427
OTU_25	Proteobacteria	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.443698
OTU_13	Proteobacteria	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.512481
OTU_941	Proteobacteria	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.561572
OTU_244	Proteobacteria	Altererythrobacter	-0.54532
OTU_214	Proteobacteria	Aminobacter	0.471688
OTU_849	Proteobacteria	Aquamicrobium	0.478735
OTU_1032	Proteobacteria	Aquicella	-0.42804
OTU_5221	Proteobacteria	Arenimonas	0.408607
OTU_690	Proteobacteria	Bauldia	-0.40214
OTU_365	Proteobacteria	Bauldia	0.614443
OTU_231	Proteobacteria	Bdellovibrio	-0.45223
OTU_495	Proteobacteria	Bdellovibrio	0.435453
OTU_37	Proteobacteria	Bosea	-0.39641
OTU_84	Proteobacteria	Bosea	0.481859
OTU_85	Proteobacteria	Bradyrhizobium	0.445319
OTU_479	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	0.568284
OTU_202	Proteobacteria	Caulobacter	0.409963
OTU_1467	Proteobacteria	Cellvibrio	-0.3977
OTU_710	Proteobacteria	Devosia	0.48285
OTU_122	Proteobacteria	Dokdonella	-0.67113
OTU_880	Proteobacteria	Dokdonella	-0.42826
OTU_215	Proteobacteria	Dongia	-0.44383
OTU_917	Proteobacteria	Ensifer	-0.40767
OTU_204	Proteobacteria	Ferrovibrio	-0.55306
OTU_406	Proteobacteria	Haliangium	0.417709
OTU_304	Proteobacteria	Haliangium	0.487205
OTU_348	Proteobacteria	Haliangium	0.502025
OTU_431	Proteobacteria	Hirschia	0.394594
OTU_101	Proteobacteria	Hydrogenophaga	-0.41081
OTU_51	Proteobacteria	Hyphomicrobium	-0.67037
OTU_1288	Proteobacteria	Hyphomicrobium	-0.66487
OTU_76	Proteobacteria	Hyphomicrobium	-0.62158
OTU_758	Proteobacteria	Hyphomicrobium	0.39234
OTU_356	Proteobacteria	Hyphomicrobium	0.513757
OTU_336	Proteobacteria	Legionella	0.42132
OTU_730	Proteobacteria	Lysobacter	0.404024
OTU_360	Proteobacteria	Lysobacter	0.498036
OTU_352	Proteobacteria	Massilia	0.422407
OTU_1216	Proteobacteria	Massilia	0.527776

OTUs	Phylum	Genus	R
OTU_74	Proteobacteria	Massilia	0.551807
OTU_103	Proteobacteria	Mesorhizobium	-0.50119
OTU_2822	Proteobacteria	Mesorhizobium	0.425973
OTU_203	Proteobacteria	Mesorhizobium	0.476046
OTU_702	Proteobacteria	Methylobacterium	0.49691
OTU_869	Proteobacteria	Methyloceanibacter	-0.45783
OTU_1443	Proteobacteria	Methylotenera	-0.5402
OTU_802	Proteobacteria	Methylotenera	-0.39136
OTU_546	Proteobacteria	Microvirga	0.405678
OTU_175	Proteobacteria	Microvirga	0.409732
OTU_1045	Proteobacteria	Microvirga	0.482123
OTU_955	Proteobacteria	MND1	-0.44471
OTU_896	Proteobacteria	Nordella	-0.43483
OTU_131	Proteobacteria	Novosphingobium	-0.59687
OTU_1514	Proteobacteria	Novosphingobium	0.433559
OTU_1512	Proteobacteria	Phenylobacterium	0.391215
OTU_1008	Proteobacteria	Phenylobacterium	0.472927
OTU_840	Proteobacteria	Phenylobacterium	0.561249
OTU_102	Proteobacteria	Pseudolabrys	-0.60139
OTU_1224	Proteobacteria	Pseudolabrys	-0.48252
OTU_1704	Proteobacteria	Pseudolabrys	0.41271
OTU_765	Proteobacteria	Pseudorhodoplanes	0.473658
OTU_1174	Proteobacteria	Ramlibacter	0.520952
OTU_662	Proteobacteria	Rhizorhapis	-0.55033
OTU_372	Proteobacteria	Rhodopseudomonas	0.555086
OTU_2364	Proteobacteria	Rhodovastum	0.396037
OTU_100	Proteobacteria	Sphingobium	-0.5733
OTU_358	Proteobacteria	Sphingobium	-0.54411
OTU_81	Proteobacteria	Sphingobium	-0.39932
OTU_459	Proteobacteria	Sphingomonas	0.426267
OTU_640	Proteobacteria	Sphingomonas	0.428877
OTU_296	Proteobacteria	Sphingomonas	0.468869
OTU_191	Proteobacteria	Sphingomonas	0.485239
OTU_282	Proteobacteria	Sphingopyxis	0.486523
OTU_145	Proteobacteria	Steroidobacter	-0.50029
OTU_1082	Proteobacteria	SWB02	-0.47876
OTU_394	Proteobacteria	SWB02	-0.44266
OTU_1223	Proteobacteria	Uni.Alphaproteobacteria	0.444893
OTU_899	Proteobacteria	Uni.Beijerinckiaceae	0.391213
OTU_978	Proteobacteria	Uni.Beijerinckiaceae	0.453351
OTU_2471	Proteobacteria	Uni.Beijerinckiaceae	0.485239
OTU_2395	Proteobacteria	Uni.Beijerinckiaceae	0.553246
OTU_238	Proteobacteria	Uni.BIrii41	-0.60447
OTU_266	Proteobacteria	Uni.BIrii41	-0.4257
OTU_419	Proteobacteria	Uni.BIrii41	0.466932

OTUs	Phylum	Genus	R
OTU_142	Proteobacteria	Uni.Burkholderiaceae	0.391062
OTU_1903	Proteobacteria	Uni.Burkholderiaceae	0.452536
OTU_616	Proteobacteria	Uni.Burkholderiaceae	0.456392
OTU_4020	Proteobacteria	Uni.Burkholderiaceae	0.470589
OTU_1007	Proteobacteria	Uni.Caulobacteraceae	0.414757
OTU_337	Proteobacteria	Uni.Cellvibrionaceae	-0.49074
OTU_3051	Proteobacteria	Uni.Diplorickettsiaceae	0.469572
OTU_588	Proteobacteria	Uni.Gammaproteobacteria	-0.52513
OTU_281	Proteobacteria	Uni.Hyphomicrobiaceae	-0.54482
OTU_578	Proteobacteria	Uni.Hyphomicrobiaceae	-0.44171
OTU_637	Proteobacteria	Uni.Methyloligellaceae	-0.55609
OTU_746	Proteobacteria	Uni.Methyloligellaceae	-0.40583
OTU_466	Proteobacteria	Uni.Micavibrionales	-0.47689
OTU_464	Proteobacteria	Uni.Micavibrionales	-0.42786
OTU_950	Proteobacteria	Uni.Micavibrionales	-0.40422
OTU_471	Proteobacteria	Uni.Micropepsaceae	0.481394
OTU_549	Proteobacteria	Uni.PLTA13	-0.50317
OTU_739	Proteobacteria	Uni.Reyranellaceae	-0.42482
OTU_421	Proteobacteria	Uni.Rhizobiaceae	-0.58596
OTU_2289	Proteobacteria	Uni.Rhizobiaceae	-0.5114
OTU_113	Proteobacteria	Uni.Rhizobiaceae	-0.4425
OTU_248	Proteobacteria	Uni.Rhizobiaceae	-0.43013
OTU_1562	Proteobacteria	Uni.Rhizobiaceae	-0.40025
OTU_111	Proteobacteria	Uni.Rhizobiaceae	0.42998
OTU_417	Proteobacteria	Uni.Rhizobiaceae	0.449084
OTU_148	Proteobacteria	Uni.Rhizobiales	-0.70379
OTU_92	Proteobacteria	Uni.Rhizobiales	-0.60891
OTU_382	Proteobacteria	Uni.Rhizobiales	-0.51785
OTU_1296	Proteobacteria	Uni.Rhizobiales	0.433043
OTU_374	Proteobacteria	Uni.Rhizobiales_Incertae_Sedis	-0.51224
OTU_209	Proteobacteria	Uni.Rhodanobacteraceae	-0.51901
OTU_389	Proteobacteria	Uni.Rhodobacteraceae	-0.56618
OTU_622	Proteobacteria	Uni.Rhodospirillales	-0.41587
OTU_400	Proteobacteria	Uni.Rhodospirillales	-0.40705
OTU_1580	Proteobacteria	Uni.Rhodospirillales	0.408626
OTU_1325	Proteobacteria	Uni.Rhodospirillales	0.449134
OTU_205	Proteobacteria	Uni.Rickettsiales	-0.49841
OTU_1105	Proteobacteria	Uni.Rickettsiales	0.443248
OTU_4448	Proteobacteria	Uni.Sandaracinaceae	0.558084
OTU_317	Proteobacteria	Uni.Sandaracinaceae	0.594079
OTU_376	Proteobacteria	Uni.Sphingomonadaceae	-0.5991
OTU_624	Proteobacteria	Uni.Sphingomonadaceae	-0.42685
OTU_2180	Proteobacteria	Uni.Sphingomonadaceae	0.461227
OTU_3590	Proteobacteria	Uni.Sphingomonadaceae	0.476439
OTU_1066	Proteobacteria	Uni.Sphingomonadaceae	0.497621

OTUs	Phylum	Genus	R
OTU_323	Proteobacteria	Uni.Sphingomonadaceae	0.508866
OTU_280	Proteobacteria	Uni.Sphingomonadaceae	0.539196
OTU_538	Proteobacteria	Uni.Xanthobacteraceae	-0.63185
OTU_303	Proteobacteria	Uni.Xanthobacteraceae	-0.54682
OTU_2066	Proteobacteria	Uni.Xanthobacteraceae	0.396282
OTU_216	Proteobacteria	Uni.Xanthobacteraceae	0.408766
OTU_1220	Proteobacteria	Uni.Xanthobacteraceae	0.4203
OTU_1485	Proteobacteria	Uni.Xanthobacteraceae	0.453222
OTU_535	Proteobacteria	Uni.Xanthobacteraceae	0.454792
OTU_2749	Proteobacteria	Uni.Xanthobacteraceae	0.46097
OTU_1365	Proteobacteria	Uni.Xanthobacteraceae	0.479836
OTU_716	Proteobacteria	Uni.Xanthobacteraceae	0.550554
OTU_1820	Proteobacteria	Uni.Xanthobacteraceae	0.592428
OTU_4025	Proteobacteria	Variovorax	0.400431
OTU_405	Proteobacteria	Variovorax	0.419367
OTU_1796	Verrucomicrobia	Alterococcus	-0.40268
OTU_768	Verrucomicrobia	Chthoniobacter	0.517364
OTU_163	Verrucomicrobia	Luteolibacter	0.394224
OTU_188	Verrucomicrobia	Luteolibacter	0.404769
OTU_1904	Verrucomicrobia	Opitutus	0.461347
OTU_901	Verrucomicrobia	Opitutus	0.55745
OTU_1252	Verrucomicrobia	Roseimicrobium	-0.41901
OTU_1412	Verrucomicrobia	Uni.Verrucomicrobiaceae	0.464875

Chapter 6

General discussion

Scope

Ecological knowledge about plants and soils can be of great importance for sustainable agriculture and agriculture is essentially applied ecology, which is the manipulation of individual organisms, populations and ecosystems to meet human needs (Weiner 2017). A main goal of plant and soil ecologists is to improve the sustainability of agricultural practices for example by reducing external energy inputs and enhancing plant and agroecosystem resistance to potential disturbances (Weiner 2017; de Vries et al. 2017; Kleijn et al. 2018). Horticultural crops suffer greatly from the threats of various above- and belowground pests, and sustainable approaches that can reduce the susceptibility of crops to pests and pathogens are urgently needed (Blom-Zandstra and Gremmen 2012; Blom-Zandstra and van Keulen 2008). Soil from natural ecosystems often harbors higher diversity and potentially more beneficial microbes than agricultural soils due to the differences in management such as the use of pesticides, fertilization and intensive cultivation practices (Mariotte et al. 2017). Plant species from natural ecosystems have also been reported to have antagonistic effects on soil-borne diseases or to promote beneficial microbes (Table 1.1). However, how soils and plants from natural ecosystems can improve the performance of crops has rarely been tested. In this thesis, I evaluated the possibilities of using wild plant species and soil from a natural ecosystem to enhance the growth and reduce the disease susceptibility of chrysanthemum through plant-soil feedback principles. Here, I will first compare the effects of inoculation with soil conditioned by wild plant species with the effects of sterilized soil on chrysanthemum growth, discuss why these effects may have been observed, and place these results in a wider context. Second, I will discuss the possibilities of reversing the negative effects of greenhouse soil after five growth cycles on chrysanthemum growth, and highlight some plant species that could be used to develop healthy soil microbiomes for chrysanthemum, and also discuss why legume soils that have been widely used in crop rotations may have strong negative effects on chrysanthemum. Third, I will discuss several microbial groups that are important for chrysanthemum performance. Finally, I will discuss ideas and directions for future studies that may help to advance our understanding of the mechanisms behind these effects.

Table 6.1 Chrysanthemum performance in un-inoculated soil and soil inoculated with plant-conditioned inocula in different treatments. The effects of plant-conditioned inocula presented in the table are the ones that showed benefits to chrysanthemum, such as *Lolium perenne*, *Festuca filiformis* or *Anthoxanthum odoratum*. Other plant-conditioned inocula (*i.e.* legume-conditioned inocula) that had strong negative effects on chrysanthemum are discussed in a later paragraph. '>' indicates chrysanthemum yields in un-inoculated soil were higher than in soil inoculated with plant-conditioned inocula. '<' indicates chrysanthemum yields in un-inoculated soil were lower than in soil with plant-conditioned inocula. 'n.s.' indicates there were no significant differences of chrysanthemum yields between un-inoculated soil and soil inoculated with plant-conditioned inocula.

10% plant-conditioned soil + 90% sterilized soil: testing the possibilities of preventing chrysanthemum from pathogen attack			
	Un-inoculated soil (100% sterilized soil)		**Plant-conditioned inocula**
Growth cycle 1			
Control	Standard yield; No leaf yellowness	**n.s.** or >	No significant increase in yield or leaf yellowness
Pythium ultimum	Yield decreased; No effects on leaf yellowness	<	Yield increased; No effects on leaf yellowness
Meloidogyne incognita	No significant effects		No significant effects
Mixing two plant-conditioned inocula			Two inocula interacted antagonistically in terms of growth, synergistically in terms of health
Growth cycle 2			
Control - Control	Yield decreased; Leaf yellowness increased	<	Yield decreased; Leaf yellowness increased
Control - Diseased soil inoculum	Yield increased; Leaf yellowness decreased	<	Yield increased; Leaf yellowness decreased
Control - *Pythium*	No significant effects	**n.s.**	No significant effects
Pythium - Control	No significant effects	**n.s.**	No significant effects
Pythium - Diseased soil inoculum	Yield increased; Leaf yellowness decreased	**n.s.**	Yield increased; Leaf yellowness decreased
Pythium - *Pythium*	Yield increased; No significant effects on leaf yellowness	**n.s.**	No effects on yield; No significant effects on leaf yellowness
Meloidogyne - Control	No significant effects	**n.s.**	No significant effects
Meloidogyne - Diseased soil inoculum	Yield increased; Leaf yellowness decreased	<	Yield increased; Leaf yellowness decreased
Meloidogyne - *Pythium*	No significant effects	**n.s.**	No significant effects
10% plant-conditioned inocula + 90% greenhouse soil: testing the possibilities of reversing the negative effects of greenhouse soil on chrysanthemum			
	Un-inoculated soil (100% greenhouse soil)		**Plant-conditioned inocula**
Chrysanthemum yield	Strong negative effects on yield	**n.s.**	Not improving yield
Pathogenic fungi	High relative abundance of pathogenic fungi	<	Relative abundance of pathogenic fungi decreased
Bacterial diversity	Low bacterial diversity	<	High bacterial diversity
Fungal diversity	No significant effects	**n.s.**	No significant effects
Growth-correlated OTUs	Both positive and negative correlated ones are increased or decreased		Both positive and negative correlated ones are increased or decreased
Performance of thrips	No significant effects	**n.s.**	No significant effects

Maximum yield vs Sustainability

Soil sterilization

Sustainability means the ability to reduce external inputs while maintaining the yield and potentially increasing the yield over time (Blom-Zandstra and Gremmen 2012). Whereas, more intensive and less sustainable horticultural practices may lead to high yields in the short-term, the yield typically decreases over time or it requires ever-increasing inputs (Blom-Zandstra and Gremmen 2012). A good example of an unsustainable horticultural practice is soil sterilization. In my studies chrysanthemum grown in sterilized soil produced the highest yield, but the yield decreased in the disease treatments or over time. Soil sterilization is a common practice used by chrysanthemum growers to eliminate soil pathogens. I observed that the yield of chrysanthemum was highest in 100% sterilized soil in several of my studies (see **Chapters 2 and 5**). However, when plants were exposed to pathogens or when the soil was used for more growth cycles, the yield in originally 100% sterilized soil dropped sharply, and the performance of chrysanthemum grown in soils inoculated with soil conditioned by wild plant species was better. For example, when exposed to *Pythium*, chrysanthemum grown with soil inocula conditioned by *Lolium perenne, Festuca* spp and *Anthoxanthum odoratum* had higher biomass than plants grown in 100% sterilized soil (**Chapter 2; Fig.S2.2A**). Moreover, in **Chapter 4** I showed that in the next growth cycle, the chrysanthemum biomass in originally 100% sterilized soil (un-inoculated soil) was lower than in other soils, indicating that addition of plant-conditioned inocula reduced the negative conspecific effects of chrysanthemum. This could be because soil sterilization leaves the soil as an empty habitat without buffering capacity, where pathogens can easily re-establish (Wei et al. 2015; van Elsas et al. 2012). Meanwhile, soil sterilization also hampers the re-establishment of the beneficial interactions between soil microbes that could protect plants from pathogen attack (Wurst and van Beersum 2009; Liu et al. 2017). In **Chapter 5** where I focused on the molecular analysis of the root-associated microbiome of chrysanthemum, I showed that the relative abundance of pathogenic fungi in roots of chrysanthemum growing in 100% sterilized soil was higher than in soils inoculated with soil conditioned by *Lolium perenne, Anthoxanthum odoratum* or *Achillea millefolium*. In **Chapter 5**, I also showed that the relative abundance of root pathogenic fungi in roots in 100% live greenhouse soil was lower than in plants growing in 10% live greenhouse soil with 90% sterilized background soil. This indicates that soil pathogens may proliferate better in sterilized soil than in soil that already contains a high abundance of pathogens. Thus, because of a lack of resistance to the pathogen invasion in sterilized soil, the high productivity of chrysanthemum that is typically found when grown in 100% sterilized soil is only observed in the first growth cycle and cannot be maintained in the longer-term.

Soil inocula conditioned by wild plant species

The effects of inoculation with plant-conditioned soil on chrysanthemum growth vary in different scenarios: First, in the control treatment (no pathogen pressure), inoculation with soil conditioned by wild plant species did not significantly increase chrysanthemum growth compared with un-inoculated soil (100% sterilized soil) (**Chapters 2 and 5**). Second, with pathogen pressure, chrysanthemum produced more biomass in sterilized soil inoculated with soil from certain plant species (*Lolium perenne* or *Anthoxanthum odoratum*) than in un-inoculated soil (100% sterilized soil) (**Chapters 2 and 4**). Here, I will discuss two potential reasons why inoculating sterilized soil with plant-conditioned soil did not immediately lead to growth promotion for chrysanthemum: the high nutrient supply in the chrysanthemum growing system and the identity of the focal plant.

Interactions between plants and soil microbiota can offer benefits to plants, such as nutrient uptake and protection from pathogen attack. Soil microbes can benefit from the carbon compounds released from plant roots (Raaijmakers et al. 2009; Philippot et al. 2013). Certain soil organisms are known to form close associations with plant species, and these symbiotic relationships are important for plants when they lack essential elements such as nitrogen or phosphorus. However, in the chrysanthemum growing system, plants receive high levels of nutrients, a situation that would negatively influence the symbiotic relationships between plants and soil microbes (Morgan et al. 2005). For example, high levels of nitrogen or phosphorus supply can directly reduce the growth and activity of mycorrhizal fungi (Oehl et al. 2004), and can inhibit the formation of symbiosis between mycorrhizal fungi and the host plant (Nouri et al. 2014; Kiernan et al. 1983). Several studies have reported that mycorrhizal fungi do colonize chrysanthemum plants (del Mar Montiel-Rozas et al. 2016; Sohn et al. 2003; D'Amelio et al. 2011). However, in **Chapter 5** I did not find mycorrhizal fungal sequences in chrysanthemum roots, thus, it is possible that in high nutrient environments, this symbiosis that could potentially benefit plant growth was inhibited. It is also possible that the benefits provided by microbes to plants may become non-significant under high nutrient supply (De Deyn et al. 2004). This has been demonstrated by several studies, for example plant growth promoting strains, such as *Pseudomonas* spp, were more efficient in promoting plant growth under low levels of nutrient supply, and their effects became non-significant under high levels of fertilization (Carlier et al. 2008; Zabihi et al. 2010). However, some bacterial strains can increase plant growth by facilitating the nutrient uptake of plants even at high nutrient supply (Miransari 2011; Shaharoona et al. 2008; Adesemoye et al. 2009). Further studies are needed to compare the effects of soil inoculation on chrysanthemum at different levels of fertilization to infer whether the current fertilization practice used in commercial chrysanthemum greenhouses overrules the potential growth promoting effects of inoculated soil communities on chrysanthemum.

Another possibility of why soil inocula conditioned by wild plant species did not show growth promoting effects in the control treatment is the focal plant I used. In a previous study (Badri et al. 2013), plants inoculated with soil microbiomes derived from other plant species showed higher biomass than in sterilized soil. This is opposite to what I observed in my studies. In that study (Badri et al. 2013), the focal plant was *Arabidopsis thaliana*, which is a wild plant species. However, chrysanthemum the species I used in my studies, is a domesticated crop. It is possible that crops, which have been selected for yield, may have a weaker ability to shape its microbiome, to sanction non-beneficial microbes, and to selectively recruit and amplify beneficial microbes compared with wild plant species (Mueller and Sachs 2015; Pérez-Jaramillo et al. 2016). Previous studies found that the rhizosphere microbiomes of domesticated crops have lower bacterial diversity (Germida and Siciliano 2001), fungal diversity (Szoboszlay et al. 2015), and more importantly a lower relative abundance of bacteria that are potentially antagonistic to soil pathogens (Pérez-Jaramillo et al. 2017) than their wild relatives, and this may make crops more susceptible to infection by soil pathogens. Recently, a study, which tested how inoculation of rhizosphere microbiomes derived from other plants on soybean and tomato alters the susceptibility to plant parasitic nematodes and growth, observed the same effects as I did in my studies (Elhady et al. 2018). They found that both crops became more resistant to plant parasitic nematodes when the soil was inoculated with rhizosphere microbiomes derived from other plants, but that these effects did not necessarily lead to an improved crop yield in the absence of diseases (Elhady et al. 2018). This study and my studies have both used crops as focal plants, thus it is possible that for domesticated crops, inoculating soils with microbiomes from other plant species could deliver benefits to the crop such as protection against pathogen attack, however, that may not improve the growth of the crop immediately.

The negative effects on chrysanthemum that are present in greenhouse soil can be reversed

In both **Chapters 4** and **5**, I collected soil from commercial chrysanthemum greenhouses that had strong negative effects on chrysanthemum growth and used this soil as diseased soil inoculum in **Chapter 4**, and as greenhouse background soil in **Chapter 5**. **Chapter 5** showed that inoculation of 10% plant-conditioned soil into 90% of this greenhouse soil did not significantly alter the negative effects of the greenhouse soil on chrysanthemum. This is opposite to the study of Mendes et al. (2011), in which addition of 10% disease suppressive soil into 90% disease conducive soil successfully changed the disease conducive soil into a disease suppressive state. It is important to note that the 10% plant-conditioned inocula used in **Chapter 5** was not soil with specific disease suppressiveness against the pathogens present in the background greenhouse soil. However, in **Chapter 4**, I found that addition of 10% diseased soil (greenhouse soil) to 90% of soil in which chrysanthemum had been grown for one cycle led to positive effects on chrysanthemum. This was observed both in soil inoculated with plant-conditioned inocula and in un-inoculated soil. One possible explanation is that this phenomenon is due to general disease suppression that developed in the soil (Schlatter et al. 2017). First, the increase in

chrysanthemum biomass of plants grown in un-inoculated soil was not only observed in the diseased soil treatment but also observed in the *Pythium* treatment in the second growth cycle. Second, one growth cycle of chrysanthemum may have resulted in increased populations of soil microbes (Bartelt-Ryser et al. 2005; Weller et al. 2002). Soil microbes can use root exudates or nutrients, and reduce the availability of the resources in the soil to pathogens (Schlatter et al. 2017). General disease suppression in soils is due to the collective competitive and antagonistic abilities of the entire community (Weller et al. 2002; Mazzola 2002). Moreover, chrysanthemum plants grown in soil inoculated with *Lolium perenne* conditioned soil increased more than grown in un-inoculated soil in the diseased soil treatment, indicating that certain plant-conditioned soils could amplify these general disease suppression effects. With the right management, general disease suppression in the soil can be enhanced and maintained for decades (Alabouvette 1986). Future studies are needed to test three questions: First, will the positive effects of general disease suppression in the soil be maintained during successive growth cycles? Second, if this is true, will soil conditioned by wild plant species amplify such positive effects? Third, is the positive influence of plant-conditioned soil on diseased soil due to the stimulation of particular (groups of) soil microbes or because of compositional shifts in the microbial community?

Plant candidates for potential use in the chrysanthemum system

The positive effects of soil conditioned by the grass *Lolium perenne* on chrysanthemum are quite consistent across all my studies. In **Chapter 2**, I found that when exposed to *Pythium*, only chrysanthemum plants grown with *Lolium perenne* conditioned inocula produced significantly higher shoot biomass than those grown in the same soil in the control treatment. In **Chapter 4**, in the second growth cycle of chrysanthemum, where diseased soil showed strong negative effects on chrysanthemum, only chrysanthemum grown in soil originally inoculated with *Lolium perenne* conditioned soil produced both higher shoot and root biomass in the diseased soil inoculum treatment. The increased chrysanthemum biomass in pots with *Lolium perenne* conditioned soil was also higher (although not significant) than the chrysanthemum biomass in new sterilized soil. Furthermore, in **Chapter 5**, *Lolium perenne* conditioned inocula also significantly influenced the relative abundance in the chrysanthemum roots of *Streptomyces* strains that were highly correlated with chrysanthemum growth: higher relative abundance of the positively correlated strain and lower relative abundance of the negatively correlated strain. Chrysanthemum grown in *Lolium perenne* conditioned soil also had lower relative abundance of root pathogenic fungi than plants grown with chrysanthemum-conditioned inocula and grown in un-inoculated soil. Compared with legumes and forbs, *Lolium perenne* was found to sustain a highly diverse microbial community in the rhizosphere (Wardle et al. 2003), and high abundance of root associated bacteria (Clayton et al. 2005). *Lolium perenne* has highly branched roots and high root biomass (Wardle et al. 1999), providing more habitat for root-associated microbes, including pathogens. Based on this, it has been suggested that *Lolium perenne* relies on the activity in the soil of bacterial species that are

antagonists to fungal pathogens (Latz et al. 2015). As the abundance of these antagonists is increased in the soil, this could subsequently benefit other plants that grow later in that same soil.

Anthoxanthum odoratum is another grass species that could be used to create a beneficial soil microbiome for chrysanthemum, especially when mixed with other inocula. Chrysanthemum grown with *Anthoxanthum odoratum* conditioned soil had higher biomass when exposed to *Pythium* than plants grown in sterilized soil (**Chapter 2**). In **Chapter 3**, chrysanthemum grown with *Anthoxanthum odoratum* conditioned soil produced the highest yield. Moreover, mixing two soil inocula on average interacted antagonistically in terms of chrysanthemum biomass, while mixing other plant-conditioned inocula with *Anthoxanthum odoratum* conditioned soil led to additive effects on chrysanthemum biomass. Although I observed a similar pattern in terms of additive effects on chrysanthemum biomass with *Festuca filiformis*, *Holcus lanatus* and *Hypochaeris radicata* conditioned inocula, the decrease in leaf yellowness of chrysanthemum was only observed when soils were mixed with *Anthoxanthum odoratum* conditioned inocula. Thus, mixing with *Anthoxanthum odoratum* conditioned inocula can provide benefits in terms of chrysanthemum health without compromising chrysanthemum growth. Previous studies found that *Anthoxanthum odoratum* stimulated the soil microbial biomass, especially the abundance of mycorrhizal fungi (De Deyn et al. 2011; Innes et al. 2004). **Chapter 3** indicated that these beneficial effects of *Anthoxanthum odoratum* on the soil would also improve the effects of other soils on chrysanthemum. Future studies are needed that test if *Anthoxanthum odoratum* conditioned soil can improve the negative effects of diseased greenhouse soil on chrysanthemum.

Strong negative effects of legume-conditioned inocula on chrysanthemum

Legumes have been widely used as cover crops because of their significant effects on fixing nitrogen (Vukicevich et al. 2016). Legumes produce high quality litter, which can lead to increases in plant biomass and increased activities of soil microbiota (Wardle et al. 2003). Moreover, legumes can also promote the abundance of mycorrhizal fungi (Scheublin et al. 2004). However, my studies showed that chrysanthemum biomass was strongly negatively influenced by legume-conditioned inocula (**Chapter 2**). Previous studies have found negative effects of leguminous plants on some beneficial bacterial groups, through the release of plant defense compounds in the rhizosphere (Latz et al. 2015; Osbourn 2003; Wubs and Bezemer 2016). However, this is not sufficient to explain the strong growth reduction caused by legume-conditioned inocula on chrysanthemum. An analysis of the composition and function of the root microbiome of a leguminous plant (*Trifolium pratense*) revealed that 70% of the root-associated microbiota consisted of the nitrogen-fixing rhizobia bacteria (Hartman et al. 2017). These nitrogen-fixing rhizobia have similar colonization processes to the host plant as pathogenic bacteria (Soto et al. 2009, 2006). When the host plant is a legume, the invading rhizobia adapt their strategies to

interact with the plant, and the leguminous host on the other hand has evolved mechanisms to discriminate rhizobia from other microbes and establish a mutualistic relationship (Soto et al. 2009). However, if the host plant species is not a legume, interacting with rhizobia may trigger a plant response similar to the infection by pathogens, which could lead to growth reduction in the host plant (Soto et al. 2009). A previous study found that the strain *Rhizobium skierniewicense* could cause crown gall disease to chrysanthemum (Puławska et al. 2012), with the infected chrysanthemum having irregular galls on the stem (Reddy 2016). Moreover, isolated *Rhizobium* strains from chrysanthemum were pathogenic to various plant species (Puławska et al. 2012). The results of **Chapter 5** showed *Rhizobium* strains that were positively and negatively correlated with chrysanthemum growth, however, the most significant growth reduction of chrysanthemum occurred between soil types, and this cannot be attributed to the difference in the relative abundance of *Rhizobium*. It is important to note that, in **Chapter 5**, chrysanthemum plants were not grown in leguminous soils. Legume-conditioned soil may have contained much higher abundance of *Rhizobium*, and also higher abundances of other nitrogen-fixing rhizobia that may have host-specificity to legumes and I speculate that these soil microbes are likely to cause growth reduction in chrysanthemum.

Important microbiota for chrysanthemum

Actinobacteria and *Firmicutes* have been well described for their roles in soil pathogen suppression (Mendes et al. 2011; Palaniyandi et al. 2013; Kim et al. 2011). **Chapter 5** showed that both *Actinobacteria* and *Firmicutes* were abundant in the roots of chrysanthemum growing in the sterilized background soil (which showed more positive effects on chrysanthemum biomass than live background soil), with the relative abundance of *Firmicutes* being especially abundant in the roots of chrysanthemum in conditioned field soil inocula with sterilized background soil. However, these two phyla were not associated with changes in chrysanthemum biomass. The relative abundance of *Chloroflexi*, *Verrucomicrobia* and *Armatimonadetes* were highest in the roots of chrysanthemum growing in the best soil combination for chrysanthemum growth and lowest in roots of chrysanthemum plants growing in the worst soil combination for growth. On the contrary, the relative abundance of *Patescibacteria* was highest in the roots of chrysanthemum growing in the worst soil combination, and lowest in the roots of chrysanthemum growing in the best soil combination. *Patescibacteria* are known to form symbiotic or parasitic lifestyles with plants, which could be costly for chrysanthemum (Sánchez-Osuna et al. 2017). Previous studies have found that *Chloroflexi* and *Verrucomicrobia* were enriched in soil with disease suppression against fungal pathogens, indicating these two phyla may be associated with disease suppressive properties of the soil (Xiong et al. 2017; Sanguin et al. 2009).

I found a high relative abundance of *Olpidium brassicae* in chrysanthemum roots when chrysanthemum was grown with live greenhouse soil, the type of soil that yielded the lowest chrysanthemum biomass. However, the relative abundance of *Olpidium brassicae* was not significantly correlated with chrysanthemum growth. *Olpidium* is known as a vector of viruses to host plants by creating wounds on the host (Campbell 1996; Raaijmakers et al. 2009). *Olpidium* does not have vector specificity to viruses, and thus it can transfer multiple viruses to many host plant species (Hiruki 1994; Teakle and Hiruki 1964). The pathogenicity of *Olpidium brassicae* to a specific plant species may depend on the presence of plant viruses, and several studies have found that the infection by *Olpidium brassicae* to some plant species did not lead to overall reduction in crop health (Bensaude 1923; Vanterpool 1990). However, other studies found that the infection by *Olpidium brassicae* led to fewer rootlets and discoloration in plant roots (Singh and Pavgi 1977). The occurrence of *Olpidium brassicae* has been well described on lettuce (Lay et al. 2018), cabbage (Singh and Pavgi 1977), groundnut (Subrahmanyam and McDonald 1980) and tobacco (Hiruki 1965). However, to our knowledge, it is the first report of the occurrence of *Olpidium brassicae* in chrysanthemum (**Chapter 5**).

Two of the eight abundant OTUs that were highly correlated with chrysanthemum growth belonged to *Streptomyces* spp, one was negatively correlated and the other one was positively correlated (**Chapter 5**), indicating that there may be an important role for *Streptomyces* spp to influence chrysanthemum growth. Clearly, a correlation between *Streptomyces* spp and chrysanthemum growth does not indicate the causal effect of *Streptomyces* spp on chrysanthemum. However, a previous study, which used the same chrysanthemum cultivar as I did, showed that *Streptomyces* strains can increase chrysanthemum growth and also protect chrysanthemum from *Pythium* infection (van der Wurff et al. 2014). *Streptomyces* are recruited actively by plants from the soil (Viaene et al. 2016). The *Streptomyces*-mediated plant growth promotion has been observed for plants like rice (Gopalakrishnan et al. 2013; 2014), wheat (Jog et al. 2014), sorghum (Gopalakrishnan et al. 2013) and tomato (Palaniyandi et al. 2014). Some of the mechanisms of plant growth promotion by *Streptomyces* include auxin production or facilitation of nutrients to plant roots (Viaene et al. 2016). *Streptomyces* strains isolated from the chrysanthemum rhizosphere soil were found to produce a significant amount of a plant growth-promoting hormone: indole acetic acid (Gajendran et al. 2012). *Streptomyces* are also known as effective biocontrol agents, and are able to produce bioactive molecules with an antagonistic effect against plant pathogens (Viaene et al. 2016). Several studies have found that certain *Streptomyces* strains were effective against pathogens such as *Fusarium* and *Rhizoctonia* (Klein et al. 2013; Cordovez et al. 2015). Moreover, *Streptomyces* can also activate plant defense against pathogen attack (Viaene et al. 2016). It is however also important to note that *Streptomyces* spp can contain phytopathogenic features, and can cause *e.g.* potato scab disease. These strains are not host specific, and can elicit scab symptoms on other plants, such as carrot or beet (Loria et al. 2006). Although **Chapter 5** showed that one *Streptomyces*

strain was highly negatively correlated with chrysanthemum growth, the scab symptom caused by phytopathogenic *Streptomyces* was not observed in chrysanthemum in my studies. Future studies are needed to isolate *Streptomyces* strains that are positively and negatively correlated with chrysanthemum growth, and re-inoculate these strains to chrysanthemum to unravel the causal effects of these strains on chrysanthemum.

Future directions

The plant microbiome is composed of active microorganisms that confer plant resistance against biotic stresses (Berg et al. 2014) and plant tolerance to abiotic stresses (Yuan et al. 2016; Santoyo et al. 2017). These functions of the microbiome of plants can be transferred to plants that lack them, such as increasing plant resistance to soil pathogens (Berg et al. 2014; Gopal et al. 2013). My studies demonstrated that the microbiome created by wild plant species growing in their native soil could be used to increase the performance of chrysanthemum. Inoculation of plant-conditioned soil into sterilized soil did not increase the chrysanthemum biomass in the absence of soil pathogens, but the highest chrysanthemum biomass was observed in soil inoculated with plant-conditioned soil when the plants were also exposed to disease treatments (**Chapters 2, 4**). It is possible that when exposed to pathogens, the abundance or activity of beneficial microbes is stimulated in the soil microbiome, these beneficial microbes could originated from the inoculated plant-conditioned soil or due to the interactions between chrysanthemum and the inoculated microbiome. Thus, a next step is to test if the high yield of chrysanthemum can be strengthened over growth cycles by selecting soil that sustained high yield of chrysanthemum from previous growth cycle, and inoculate this soil with the new relevant plant-conditioned inocula, then grow chrysanthemum in it. Swenson et al (2000) and Panke-Buisse et al (2015) have successfully used artificial selection for host microbiomes with desired functions. After initial soil sterilization, they inoculated plants with a starter soil microbiome. At the end of each growth cycle, a host trait was evaluated for each soil replicate, such as plant biomass or flowering time. Then the soil from the best performing plant was used to inoculate again into sterilized soil and a next generation of plants was grown in this soil. However, this microbiome selection is a time consuming process. In both studies, ten rounds of selection for soil microbiomes produced significantly different plant phenotypes (Swenson et al. 2000; Panke-Buisse et al. 2015). In their studies, the starter microbiome arose from the microbiome of the same plant species. However, in my studies, I inoculated chrysanthemum with microbiomes derived from other plant species, and significant differences in terms of chrysanthemum growth and health were observed only after one growth cycle (**Chapter 2**). Based on their studies, I propose an artificial selection procedure for selecting chrysanthemum rhizosphere microbiomes that could lead to higher chrysanthemum growth and better resistance to soil pathogens (Fig.6.1).

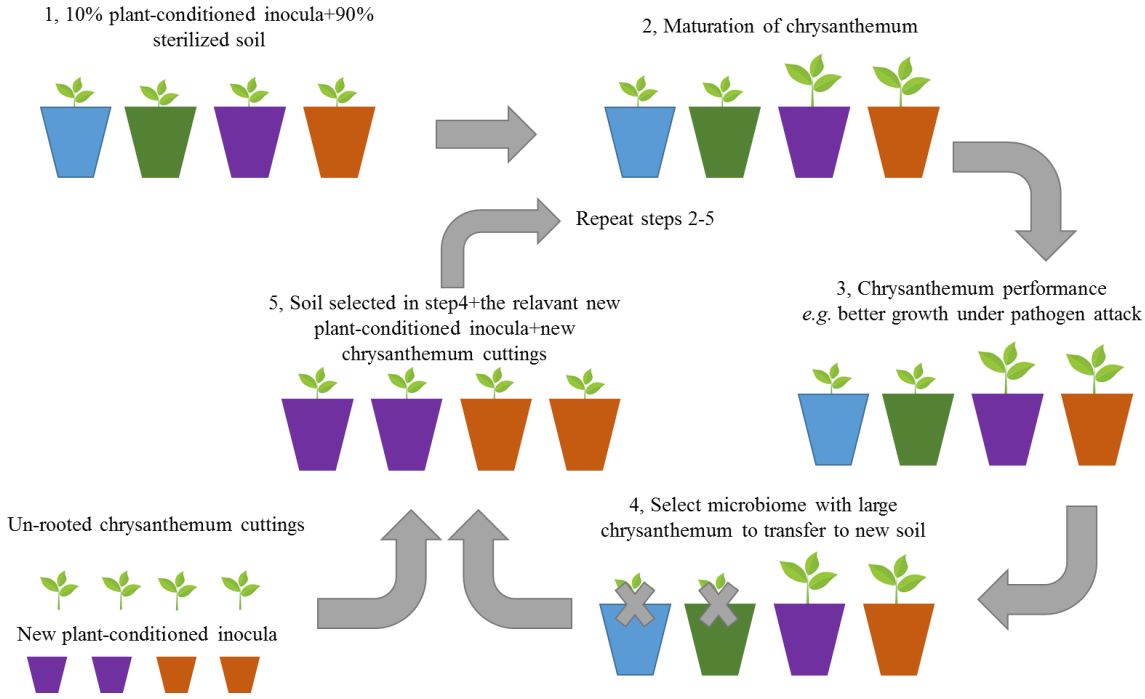

Fig. 6.1. Selection on microbiomes that produce best chrysanthemum performance. Chrysanthemums are inoculated with 10% inocula conditioned by different wild plant species + 90% sterilized soil (Step 1). The host-microbiome associations are allowed to mature (Step2), pathogen treatments can be added at this step to select for microbiomes that increase resistance of chrysanthemum against pathogens. Then, based on the performance of new chrysanthemum plants, microbiomes are chosen to inoculate during the next generation (Step 3, 4). New chrysanthemum cuttings will be rooted in the selected soils from the previous round with the new relevant plant-conditioned inoculum (Step 5), and step 2-5 will be repeated until the microbiome with the best performance of chrysanthemum is produced. Scheme modified from Mueller and Sachs 2015.

Based on the context of this thesis, I see another important area for future research: the need to establish the link between 'the inoculated soil community' and 'the established soil community'. This means to unravel the soil microbial community in the soil inocula conditioned by wild plant species, and which parts or groups from this soil community successfully establish in, on, or around chrysanthemum roots and subsequently influence the growth of this plant species. More importantly, to determine the rules by which microbes from the inoculated microbiome assemble into the root microbiome of chrysanthemum. This is essential for future attempts to manipulate and manage the microbiome of chrysanthemum (Busby et al. 2017). A recent study, which compared the root microbiomes of 30 angiosperm species, found that greater similarities among the root microbiomes between hosts led to more negative plant-soil feedback effects (Fitzpatrick et al. 2018). Thus, it is possible that the wild plant species with the most different root microbiome compared with chrysanthemum, will have the most positive feedback effect on chrysanthemum. However, several studies also found that crops can benefit from microbiomes of their wild relatives (Santhanam et al. 2015; Pérez-Jaramillo et al. 2018). Therefore, the relationship

of the similarity between the root microbiomes of plants and their plant-soil feedback effects may vary in different target plant species. Moreover, our understanding of the relationship between the soil microbes that were inoculated and the ones that establish should move beyond similarities between these microbiomes. Specifically, I see four main research questions that should be answered in future studies. First, which part of the microbiome from the soil inocula conditioned by wild plant species establishes in chrysanthemum root environments? Second, what is the difference between the abundance of the established microbes in their original soil community compared with the new soil community (*i.e.* does chrysanthemum selectively increase the population of some microbes from the inoculated soil community, and will the populations of other microbes be inhibited by chrysanthemum)? Third, to identify beneficial and pathogenic microbes of chrysanthemum, and to determine how much of these microbes are from the inoculated soil community and how much are carried by chrysanthemum itself? Fourth, using manipulative methods, how do the established microbes influence beneficial and pathogenic microbes that are essential to chrysanthemum?

A promising direction in plant-soil feedbacks is to use plant species and soil from natural ecosystems to create effective soil microbiomes that suppress soil pathogens in chrysanthemum. Previous studies have found that soil immunity, which is natural disease suppression, can be induced in agricultural soils that have continuous (decades) mono-cropping and after a severe outbreak of certain soil-borne diseases (Schlatter et al. 2017; Raaijmakers and Mazzola 2016). However, to simulate this in the chrysanthemum greenhouse is not feasible since during the process (and hence exposure to pathogens), there will be severe economic loss. Moreover, not every long-term mono-cropping system would develop natural disease suppression in the soil. Alternatively, specialized soil disease suppressive microbiomes could be created by exposing wild plant species to pathogens of chrysanthemum when growing in their native soil in the conditioning phase. This is due to: first, soil-borne diseases of chrysanthemum, such as *Pythium, Meloidogyne* or *Olpidium,* have a broad range of host plants, and are also present in natural ecosystems (Kageyama 2014; Renčo and Murín 2013; Lay et al. 2018). Thus, plant species from natural ecosystems may have evolved mechanisms to defend themselves against pathogen infections. Second, wild plant species that showed positive effects on chrysanthemum through influencing the soil, such as *Lolium perenne* and *Anthoxanthum odoratum*, have been reported to be less susceptible to *Pythium* or *Meloidogyne* compared with forb or other grasses (Bithell et al. 2011; Mills and Bever 1998; Stiles et al. 2007). Therefore, when encountering soil pathogens, *Lolium perenne* and *Anthoxanthum odoratum* may be more effective in increasing the population of beneficial soil microbes in the soil, eventually leading to a complete shift in the composition of their rhizosphere microbiome (van Dam 2009; Wei et al. 2015). Analog to vaccines for humans, which stimulate the immune system by weakened pathogens, a disease suppressive soil microbiome would also be created by stimulating plant species that have strong defense against pathogens. Inoculation with these soils could then protect chrysanthemum from

the infection of pathogens. However, a balance should be established between stimulating the formation of a disease suppressive soil microbiome and avoiding remnant pathogens in this soil microbiome by testing different concentrations of pathogens during the conditioning phase. Future studies are needed to test the possibility of creating such defense-oriented microbiomes by exposing wild plant species to the specific pathogens. It is important to note that these plant species need to be grown in their native soils when exposing to pathogens, because in **Chapter 5**, when I grew wild plant species directly in the chrysanthemum greenhouse soil, no significant effects on chrysanthemum performance were observed.

Conclusions

In this thesis, I show that wild plant species and soil from natural ecosystems can be used to improve chrysanthemum performance through plant-soil feedback effects. Soil inoculum that originated from wild plant species had strong species-specific and functional group effects on chrysanthemum growth. Grass-conditioned inocula contributed more to chrysanthemum growth than forb- or legume-conditioned inocula. Chrysanthemum grown in soil conditioned by some grass, such as *Lolium perenne*, *Anthoxanthum odoratum*, showed higher biomass than in 100% sterilized soil under *Pythium* pressure. Moreover, by mixing two plant-conditioned inocula, on average, they interacted synergistically in terms of plant health but antagonistically in terms of plant growth. The influence of plant-conditioned inocula on chrysanthemum were still significant after two successive growth cycles, but their effects tended to converge because of the overriding effect of chrysanthemum growth. Inoculating greenhouse soil conditioned by wild plant species or grassland soil to greenhouse soil did not significantly improve the negative effects of greenhouse soil on chrysanthemum growth. However, growing certain wild plant species in greenhouse soil and then inoculating this soil decreased the relative abundance of pathogenic fungi in chrysanthemum roots. Overall, my studies highlight the potential of using resources from natural ecosystems to enhance sustainability in horticulture and future studies should examine how plant-soil feedback principles can be implemented in commercial horticultural systems.

References

Abarenkov, K., Henrik Nilsson, R., Larsson, K.H., Alexander, I.J., Eberhardt, U., Erland, S., and Sen, R. 2010. The UNITE database for molecular identification of fungi–recent updates and future perspectives. New Phytologist 186:281-285.

Adesemoye, A.O., and Kloepper, J.W. 2009. Plant–microbes interactions in enhanced fertilizer-use efficiency. Applied Microbiology and Biotechnology 85:1-12.

Agrios, G.N. 2005. Plant Pathology. Orlando: Academic press.

Alabouvette, C. 1986. *Fusarium*-wilt suppressive soils from the Châteaurenard region: review of a 10-year study. Agronomie 6:273-284.

Alabouvette, C., Olivain, C., Migheli, Q., and Steinberg, C. 2009. Microbiological control of soil‐borne phytopathogenic fungi with special emphasis on wilt‐inducing *Fusarium oxysporum*. New Phytologist 184:529-544.

Alizadeh, H., Behboudi, K., Ahmadzadeh, M., Javan-Nikkhah, M., Zamioudis, C., Pieterse, C.M., and Bakker, P. A. 2013. Induced systemic resistance in cucumber and *Arabidopsis thaliana* by the combination of *Trichoderma harzianum* Tr6 and *Pseudomonas* sp. Ps14. Biological Control 65:14-23.

Allison, S.D., and Martiny, J.B. 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences 105:11512-11519.

Amossé, C., Jeuffroy, M.H., Mary, B., and David, C. 2014. Contribution of relay intercropping with legume cover crops on nitrogen dynamics in organic grain systems. Nutrient Cycling in Agroecosystems 98:1-14.

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

Anyango, J.J. 1992. The life history of chrysanthemum thrips, Thrips *nigropilosus* Uzel (Thripidae: Thysanoptera) and an evaluation of the effects of temperature on its life cycle. International Journal of Tropical Insect Science 13:41-44.

Arabidopsis Genome Initiative. (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796-815.

Awasthi, L.P. 2015. Recent advances in the diagnosis and management of plant diseases. Springer.India.

Badenes-Pérez, F.R., and López-Pérez, J.A. 2018. Resistance and susceptibility to powdery mildew, root-knot nematode, and western flower thrips in two types of winter cress (Brassicaceae). Crop Protection 110:41-47.

Badri, D.V., Weir, T.L., van der Lelie, D., and Vivanco, J.M. 2009. Rhizosphere chemical dialogues: plant–microbe interactions. Current Opinion in Biotechnology 20:642-650.

Badri, D.V., Zolla, G., Bakker, M.G., Manter, D.K., and Vivanco, J.M. 2013. Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. New Phytologist 198:264-273.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., and Vivanco, J.M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology 57:233-266.

Bakker, M.G., Schlatter, D.C., Otto-Hanson, L., and Kinkel, L.L. 2014. Diffuse symbioses: roles of plant–plant, plant–microbe and microbe–microbe interactions in structuring the soil microbiome. Molecular Ecology 23:1571-1583.

Bakker, P.A.H.M, Berendsen, R.L., Doornbos, R.F., Wintermans, P.C.A., and Pieterse, C.M.J. 2013. The rhizosphere revisited: root microbiomics. Frontiers in Plant Science 4:165.

Bakker, P.A.H.M., Pieterse, C.M.J., de Jonge, R., and Berendsen, R.L. 2018. The soil-borne legacy. Cell 172:1178-1180.

Barberán, A., Bates, S.T., Casamayor, E.O., and Fierer, N. 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. The ISME Journal 6:343-351.

Bardgett, R.D., and Wardle, D.A. 2010. Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change. New York: Oxford University Press.

Bartelt-Ryser, J., Joshi, J., Schmid, B., Brandl, H., ann 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.

Baxendale, C., Orwin, K.H., Poly, F., Pommier, T., and Bardgett, R.D. 2014. Are plant-soil feedback responses explained by plant traits? New Phytologist 204:408-423.

Bayer, C., Dieckow, J., Amado, T.J.C., Eltz, F.L.F., and Vieira, F.C.B. 2009. Cover crop effects increasing carbon storage in a subtropical no‐till sandy Acrisol. Communications in Soil Science and Plant Analysis 40:1499-1511.

Becklin, K.M., Hertweck, K.L., and Jumpponen, A. 2012. Host identity impacts rhizosphere fungal communities associated with three alpine plant species. Microbial Ecology 63:682-693.

Bensaude, M., 1923. A species of *Olpidium* parasitic in the roots of tomato, tobacco and cabbage. Phytopathology 13:451-454.

Berendsen R.L., Pieterse C.M.J., Bakker P.A.H.M. 2012. The rhizosphere microbiome and plant health. Trends in Plant Science 17:478-486.

Berendsen, R.L., Vismans, G., Yu, K., Song, Y., Jonge, R., Burgman, W.P., Burmølle, M., Herschend, J., Bakker, P.A.H.M., and Pieterse, C.M.J. 2018. Disease-induced assemblage of a plant-beneficial bacterial consortium. The ISME Journal 12:1496-1507.

Berg, G. 2009. Plant–microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. Applied Microbiology and Biotechnology 84:11–18.

Berg, G., Grube, M., Schloter, M., and Smalla, K. 2014. Unraveling the plant microbiome: looking back and future perspectives. Frontiers in Microbiology 5:148.

Berg, G., Roskot, N., Steidle, A., Eberl, L., Zock, A., and Smalla, K. 2002. Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different *Verticillium* host plants. Applied and Environmental Microbiology 68:3328-3338.

Bertalan, M., Albano, R., de Pádua, V., Rouws, L., Rojas, C., Hemerly, A., et al 2009. Complete genome sequence of the sugarcane nitrogen-fixing endophyte *Gluconacetobacter diazotrophicus* Pal5. BMC Genomics 10:450.

Bessler, H., Temperton, V.M., Roscher, C., Buchmann, N., Schmid, B., Schulze, E., Weisser, W.W., and Engels, C. 2009. Aboveground overyielding in grassland mixtures is associated with reduced biomass partitioning to belowground organs. Ecology 90:1520-1530.

Bever, J.D., Westover, K.M., and Antonovics, J. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology 85:561-573.

Bezemer T.M., Jing J., Bakx‐Schotman J.M.T., and Bijleveld, E. 2018. Plant competition alters the temporal dynamics of plant‐soil feedbacks. Journal of Ecology 106:2287-2300.

Bezemer, T.M., De Deyn, G.B., Bossinga, T.M., van Dam, N.M., Harvey, J.A., and van der Putten, W.H. 2005a. Soil community composition drives aboveground plant-herbivore-parasitoid interactions. Ecology Letters 8:652-661.

Bezemer, T.M., and van Dam, N.M. 2005b. Linking aboveground and belowground interactions via induced plant defenses. Trends in Ecology and Evolution 20:617-624.

Bezemer, T.M., Lawson, C.S., Hedlund, K., Edwards, A.R., Brook, A.J., Igual, J.M., Mortimer, S.R., and 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.

Bezemer, T.M., van der Putten, W.H., Martens, H., van de Voorde, T.F.J., Mulder, P.P., and Kostenko, O. 2013. Above-and below-ground herbivory effects on below-ground plant-fungus interactions and plant-soil feedback responses. Journal of Ecology 101:325-333.

Bharadwaj, D.P., Lundquist, P.O., and Alström, S. 2007. Impact of plant species grown as monocultures on sporulation and root colonization by native arbuscular mycorrhizal fungi in potato. Applied Soil Ecology 35:213-225.

Binkley, D., and Giardina, C. 1998. Why do trees species affectsoils? The warp and woof of tree-soil interactions. Biogeochemistry 42:89–106.

Bithell, S.L., Butler, R.C., Harrow, S., McKay, A., and Cromey, M.G. 2011. Susceptibility to take‐all of cereal and grass species, and their effects on pathogen inoculum. Annals of Applied Biology 159:252-266.

Blom-Zandstra, M., and Gremmen, B. 2012. Comparison of management styles in organic and conventional farming with respect to disruptive external influences. The case of organic dairy farming and conventional horticulture in the Netherlands. Journal of Sustainable Agriculture 36:893-907.

Blom-Zandstra, M., and Van Keulen, H. 2008. Innovative concepts towards sustainability in organic horticulture: testing a participatory technology design. International Journal of Agricultural Sustainability 6:195-207.

Brandt, A.J., de Kroon, H., Reynolds, H.L., and Burns, J.H. 2013. Soil heterogeneity generated by plant-soil feedbacks has implications for species recruitment and coexistence. Journal of Ecology 101:277-286.

Brandt, A.J., Seabloom, E.W., and Hosseini, P.R. 2009. Phylogeny and provenance affect plant-soil feedbacks in invaded California grasslands. Ecology 90:1063-1072.

Brant, V., Neckář, K., Pivec, J., Duchoslav, M., Holec, J., Fuksa, P., and Venclová, V. 2009. Competition of some summer catch crops and volunteer cereals in the areas with limited precipitation. Plant Soil and Environment 55:17-24.

Braun-Kiewnick, A., Jacobsen, B.J., and Sands, D.C. 2000. Biological control of *Pseudomonas syringae* pv. *syringae*, the causal agent of basal kernel blight of barley, by antagonistic *Pantoea agglomerans*. Phytopathology 90:368-375.

Brinkman, E.P., van der Putten, W.H., Bakker, E., and Verhoeven, K.J.F. 2010. Plant–soil feedback: experimental approaches, statistical analyses and ecological interpretations. Journal of Ecology 98:1063-1073.

Busby, P.E., Soman, C., Wagner, M.R., Friesen, M.L., Kremer, J., Bennett, A., Morsy, M., Eisen, J.A., Leach, J.E. and Dangl, J.L. 2017. Research priorities for harnessing plant microbiomes in sustainable agriculture. PLoS Biology 15:e2001793.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E.V.L., and Schulze-Lefert, P. 2013. Structure and functions of the bacterial microbiota of plants. Annual Review of Plant Biology 64:807-838.

Burns, J.H., Brandt, A.J., Murphy, J.E., Kaczowka, A.M., and 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.

Burns, J.H., and Strauss, S.Y. 2011. More closely related species are more ecologically similar in an experimental test. Proceddings of the National Academy of Sciences of the United States of America 108:5302-5307.

Campbell, R.N. 1996. Fungal transmission of plant viruses. Annual Review of Phytopathology 34:87-108.

Cao, L., Qiu, Z., You, J., Tan, H., and Zhou, S. 2004. Isolation and characterization of endophytic *Streptomyces* strains from surface‐sterilized tomato (*Lycopersicon esculentum*) roots. Letters in Applied Microbiology 39:425-430.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., and Knight, R. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal 6:1621-1624.

Carbajo, V., Den Braber, B., Van Der Putten, W.H., and De Deyn, G.B. 2011. Enhancement of late successional plants on ex-arable land by soil inoculations. PloS one 6:e21943.

Cardinale, B.J., Wright, J.P., Cadotte, M.W., Carroll, I.T., Hector, A., Srivastava, D.S., Loreau, M., and Weis, J.J. 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. Proceddings of the National Academy of Sciences of the United States of America 104:18123-18128.

Carlier, E., Rovera, M., Jaume, A.R., and Rosas, S.B. 2008. Improvement of growth, under field conditions, of wheat inoculated with *Pseudomonas chlororaphis* subsp. *aurantiaca* SR1. World Journal of Microbiology and Biotechnology 24:2653-2658.

Carmona, D., Lajeunesse, M.J., and Johnson, M.T. 2011. Plant traits that predict resistance to herbivores. Functional Ecology 25:358-367.

Castillo P, Mora-Rodríguez, M.P., Navas-Cortés, J.A., and Jiménez-Díaz, R.M. 1998. Interactions of *Pratylenchus thornei* and *Fusarium oxysporum* f. sp. ciceris on chickpea. Phytopathology 88:828-836.

Chang, C., Fu, X., Zhou, X., Guo, M., and Wu, F. 2017. Effects of seven different companion plants on cucumber productivity, soil chemical characteristics and *Pseudomonas* community. Journal of Integrative Agriculture 16:2206-2214.

Chang, C., Zhou, X., Fu, X., Yang, S., and Wu, F. 2016. Soil enzymes and bacterial community composition in cucumber (*Cucumis sativus* L.) monocropping and companion cropping systems. Allelopathy Journal 38:133-146.

Chaparro, J.M., Sheflin, A.M., Manter, D.K., and Vivanco, J.M. 2012. Manipulating the soil microbiome to increase soil health and plant fertility. Biolofy and Fertility of Soils 48:489-499.

Chaparro, J.M., Badri, D.V., and Vivanco, J.M. 2014. Rhizosphere microbiome assemblage is affected by plant development. The ISME Journal 8:790-803.

Chen, D., Pan, Q., Bai, Y., Hu, S., Huang, J., Wang, Q., Naeem, S., Elser, J.J., Wu, J., and Han, X. 2016. Effects of plant functional group loss on soil biota and net ecosystem exchange: a plant removal experiment in the Mongolian grassland. Journal of Ecology 104:734-743.

Chen, M., Chen, B., and Marschner, P. 2008. Plant growth and soil microbial community structure of legumes and grasses grown in monoculture or mixture. Journal of Environental Sciences 20:1231-1237.

Cheng, X., and Baumgartner, K. 2004. Arbuscular mycorrhizal fungi-mediated nitrogen transfer from vineyard cover crops to grapevines. Biology and Fertility of Soils 40:406-412.

Chitwood, D.J. 2002. Phytochemical based strategies for nematode control. Annual Review of Phytopathology, 40:221–249.

Clayton, S.J., Clegg, C.D., Murray, P.J., and Gregory, P.J. 2005. Determination of the impact of continuous defoliation of *Lolium perenne* and *Trifolium repens* on bacterial and fungal community structure in rhizosphere soil. Biology and Fertility of Soils 41:109-115.

Cordovez, V., Carrion, V.J., Etalo, D.W. Mumm, R., Zhu, H., van Wezel, G.P., and Raaijmakers, J.M. 2015. Diversity and functions of volatile organic compounds produced by *Streptomyces* from a disease-suppressive soil. Frontier in Microbiology 6:1081.

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

Costa, R., Götz, M., Mrotzek, N., Lottmann, J., Berg, G., and Smalla, K. 2006. Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. FEMS Microbiology Ecology 56:236-249.

Costello, M.J. 2010. Growth and yield of cultivated grape with native perennial grasses nodding needlegrass or California barley as cover crops. HortScience 45:154-156.

D'Amelio, R., Berta, G., Gamalero, E., Massa, N., Avidano, L., Cantamessa, S., D'Agostino, G., Bosco, D., and Marzachı`, C. 2011. Increased plant tolerance against chrysanthemum yellows phytoplasma ('*Candidatus*

Phytoplasma asteris') following double inoculation with *Glomus mosseae* BEG12 and *Pseudomonas putida* S1Pf1Rif. Plant Pathology 60:1014-1022.
Dastgheib, F., Kumar, K., and Goh, K.M. 1999. Weed infestations in wheat cropping systems as affected by crop residues and their management practices. Biological Agriculture & Horticulture 16:395-407.
De Deyn, G.B., Quirk, H., Yi, Z., Oakley, S., Ostle, N.J., and Bardgett, R.D. 2009. Vegetation composition promotes carbon and nitrogen storage in model grassland communities of contrasting soil fertility. Journal of Ecology 97:864-875.
De Deyn, G.B., Quirk, H., and Bardgett, R.D. 2011. Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. Biology Letters 7:75-78.
De Deyn, G.B. 2017. Plant life history and above-belowground interactions: missing links. Oikos 126:497-507.
De Deyn, G.B., Quirk, H., and Bardgett, R.D. 2010. Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. Biology Letters 7:75–78.
De Deyn, G.B., Raaijmakers, C.E., and van der Putten, W.H. 2004. Plant community development is affected by nutrients and soil biota. Journal of Ecology 92:824-834.
de Hollander M. 2017. nioo-knaw/hydra: Version 1.3.3. Zenodo. [WWW document] URL http://doi.org/10.5281/zenodo.884028 [accessed 04 September 2017].
de la Peña, E., Baeten, L., Steel, H., Viaene, N., De Sutter, N., De Schrijver, A., and Verheyen, K. 2016. Beyond plant–soil feedbacks: mechanisms driving plant community shifts due to land-use legacies in post-agricultural forests. Functinal Ecology 30:1073-1085.
de Ridder-Duine, A.S., Kowalchuk, G.A., Gunnewiek, P.J.K., Smant, W., van Veen, J.A., and de Boer, W. 2005. Rhizosphere bacterial community composition in natural stands of *Carex arenaria* (sand sedge) is determined by bulk soil community composition. Soil Biology and Biochemistry 37:349-357.
de Vries, F.T., and Bardgett, R.D. 2012. Plant–microbial linkages and ecosystem nitrogen retention: lessons for sustainable agriculture. Frontiers in Ecology and the Environment 10:425-432.
de Vries, F.T., and Wallenstein, M.D. 2017. Below-ground connections underlying above-ground food production: a framework for optimising ecological connections in the rhizosphere. Journal of Ecology 105:913-920.
del Mar Montiel-Rozas, M., López-García, Á., Kjøller, R., Madejón, E., and Rosendahl, S. 2016. Organic amendments increase phylogenetic diversity of arbuscular mycorrhizal fungi in acid soil contaminated by trace elements. Mycorrhiza 26:575-585.
Detheridge, A.P., Brand, G., Fychan, R., Crotty, F.V., Sanderson, R., Griffith, G.W., and Marley, C.L. 2016. The legacy effect of cover crops on soil fungal populations in a cereal rotation. Agriculture Ecosystem and Environment 228:49-61.
Devrnja, N., Anđelković, B., Aranđelović, S., Radulović, S., Soković, M., Krstić-Milošević, D., Ristić, M., and Ćalić, D. 2017. Comparative studies on the antimicrobial and cytotoxic activities of *Tanacetum vulgare* L. essential oil and methanol extracts. South African Journal of Botany 111:212-221.
Dhandaydham, M., Charles, L., Zhu, H., Starr, J.L., Huguet, T., Cook, D.R., Prosperi, J., and Opperman, C. 2008. Characterization of root-knot nematode resistance in *Medicago truncatula*. Journal of Nematology 40:46-54.
Dias, T., Dukes, A., and Antunes, P.M. 2015. Accounting for soil biotic effects on soil health and crop productivity in the design of crop rotations. Science of Food and Agriculture 95:447-454.
Dias, W.P., Moraes, L.A.C., de Carvalho, C.G.P., de Oliveira, M.C.N., Orsini, I.P., and de Leite, R.M.V.B.C. 2016. Resistance to *Meloidogyne incognita*, *Meloidogyne javanica* and *Pratylenchus brachyurus* in sunflower cultivars adapted to the tropical region of Brazil. Tropical Plant Pathology, 41:325-330.
Dietz, M., Machill, S., Hoffmann, H.C., and Schmidtke, K. 2013. Inhibitory effects of *Plantago lanceolata* L. on soil N mineralization. Plant and Soil 368:445-458.
Dignam, B.E., O'Callaghan, M., Condron, L.M., Raaijmakers, J.M., Kowalchuk, G.A., and Wakelin, S.A. 2016. Challenges and opportunities in harnessing soil disease suppressiveness for sustainable pasture production. Soil Biology and Biochemistry 95:100-111.
Drinkwater, L.E., Wagoner, P., and Sarrantonio, M. 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. Nature 396:262-265.
Druzhinina, I.S., Seidl-Seiboth, V., Herrera-Estrella, A., Horwitz, B.A., Kenerley, C.M., Monte, E., Mukherjee, P.K., Zeilinger, S., Grigoriev, I.V., and Kubicek, C.P. 2011. *Trichoderma*: the genomics of opportunistic success. Nature Reviews Microbiology 9:749-759.
Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N.K., Bhatnagar, S., Eisen, J.A., and Sundaresan, V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. Proceedings of the National Academy of Sciences 112:911-920.
Ehrenfeld, J.G., Ravit, B., and Elgersma, K. 2005. Feedback in the plant-soil system. Annual Review of Environment and Resources 30:75-115.
Elhady, A., Adss, S., Hallmann, J., and Heuer, H. 2018. Rhizosphere microbiomes modulated by pre-crops assisted plants in defense against plant-parasitic nematodes. Frontiers in Microbiology 9:1133.

Estendorfer, J., Stempfhuber, B., Haury, P., Vestergaard, G., Rillig, M. C., Joshi, J., Schröder, P., and Schloter, M. 2017. The Influence of Land Use Intensity on the Plant-Associated Microbiome of *Dactylis glomerata* L. Frontiers in Plant Science 8:930.

Fitzpatrick, C.R., Copeland, J., Wang, P.W., Guttman, D.S., Kotanen, P.M., and Johnson, M.T.J. 2018. Assembly and ecological function of the root microbiome across angiosperm plant species. Proceedings of the National Academy of Sciences 115:1157-1165.

Foster, R.C., Rovira, A.D., and Cock, T.W. 1983. Ultrastructure of the root-soil interface. American Phytopathological Society.

Francioli, D., Schulz, E., Lentendu, G., Wubet, T., Buscot, F., and Reitz, T. 2016. Mineral vs. organic amendments: microbial community structure, activity and abundance of agriculturally relevant microbes are driven by long-term fertilization strategies. Frontiers in Microbiology 7:1446.

Franco, C.M., Araujo, R., Adetutu, E., Tobe, S.S., Mallya, S., Paul, B., and Satyamoorthy, K. 2016. Complete genome sequences of the endophytic *Streptomyces* strains EN16, EN23, and EN27, isolated from wheat plants. Genome Announcements 4:e01342-16.

Gadhave, K.R., Hourston, J.E., and Gange, A.C. 2016. Developing soil microbial inoculants for pest management: can one have too much of a good thing?. Journal of Chemical Ecology 42:348-356.

Gajendran, S., Jeyanthi Rebecca, L., and Sharmila, S.A. 2012. Study on the Indole Acetic Acid Production by *Streptomyces* spp. Isolated from the Rhizosphere Soil of the Five Flowering Plants. International Journal of Agriculture and Food Science Technology 3:21-27.

Galletti, S., Sala, E., Leoni, O., Burzi, P.L., and Cerato, C. 2008. *Trichoderma* spp. tolerance to *Brassica carinata* seed meal for a combined use in biofumigation. Biological Control 45:319-327.

Garbeva, P., Postma, J., van Veen, J.A., and van Elsas, J.D. 2006. Effect of above-ground plant species on soil microbial community structure and its impact on suppression of *Rhizoctonia solani* AG3. Environmental Microbiology 8:233-246.

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

Gebhardt, M., Fehmi, J.S., Rasmussen, C., and Gallery, R.E. 2017. Soil amendments alter plant biomass and soil microbial activity in a semi-desert grassland. Plant and Soil, 419:53-70.

Germida, J., and Siciliano, S. 2001. Taxonomic diversity of bacteria associated with the roots of modern, recent and ancient wheat cultivars. Biology and Fertility of Soils 33:410-415.

Gerson, U., and Weintraub, P.G. 2007. Mites for the control of pests in protected cultivation. Pest Management Science: Formerly Pesticide Science 63:658-676.

Gianinazzi, S., Gollotte, A., Binet, M.N., van Tuinen, D., Redecker, D., and Wipf, D. (2010). Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20:519-530.

Gilbert, G.S., and Webb, C.O. 2007. Phylogenetic signal in plant pathogen-host range. Proceedings of the National Academy of Sciences 104, 4979-4983.

Goar, L.G. 1934. Vetches and related crops for forage. USDA, Folleto.

Gómez Expósito, R., Bruijn, I.D., Postma, J., and Raaijmakers, J.M. 2017. Current insights into the role of rhizosphere bacteria in disease suppressive soils. Frontier in Microbiology 8:2529.

Gopal, M., Gupta, A., and Thomas, G. V. 2013. Bespoke microbiome therapy to manage plant diseases. Frontiers in Microbiology 4:355.

Gopalakrishnan, S., Vadlamudi, S., Bandikinda, P., Sathya, A., Vijayabharathi, R., Rupela, O., Kudapa, H., Katta, K., and Varshney, R.K. 2014. Evaluation of *Streptomyces* strains isolated from herbal vermicompost for their plant growth-promotion traits in rice. Microbiological Research 169:40-48.

Gopalakrishnan, S., Srinivas, V., Vidya, M.S., and Rathore, A. 2013. Plant growth-promoting activities of *Streptomyces* spp. in sorghum and rice. SpringerPlus 2:574.

Griffiths, B.S., and Philippot, L. 2013. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiology Reviews 37:112-129.

Gweon, H.S., Oliver, A., Taylor, J., Booth, T., Gibbs, M., Read, D.S., Griffiths, R.I., and Schonrogge, K. 2015. PIPITS: an automated pipeline for analyses of fungal internal transcribed spacer sequences from the Illumina sequencing platform. Methods in Ecology and Evolution 6:973-980.

Haas, D., and Défago, G. 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nature Reviews Microbiology, 3:307-319.

Hagan, A., Gazaway, W., and Sikora, E. 1998. Nematode suppressive crops. The Alabama Cooperative Extension System. Sikora, RA (ed.). Alabama A&M University and Auburn University, ANR, 856.

Hajihassani, A., Maafi, Z.T., and Hosseininejad, A. 2013. Interactions between *Heterodera filipjevi* and *Fusarium culmorum*, and between *H. filipjevi* and *Bipolaris sorokiniana* in winter wheat. Journal of Plant Disease and Protection 120:77-84.

Hamid, M.I., Hussain, M., Wu, Y., Zhang, X., Xiang, M., and Liu, X. 2017. Successive soybean-monoculture cropping assembles rhizosphere microbial communities for the soil suppression of soybean cyst nematode. Frontier in Microbiology 93:1.

Harguindeguy, N.P., Blundo, C.M., Gurvich, D.E., Díaz, S., and Cuevas, E. 2008. More than the sum of its parts? Assessing litter heterogeneity effects on the decomposition of litter mixtures through leaf chemistry. Plant and Soil 303:151-159.
Harris, J. 2009. Soil microbial communities and restoration ecology: facilitators or followers? Science 325:573-574.
Harrison, K.A., and Bardgett, R.D. 2010. Influence of plant species and soil conditions on plant-soil feedback in mixed grassland communities. Journal of Ecology 98:384-395.
Hartman, K., van der Heijden, M.G.A., Wittwer, R.A., Banerjee, S., Walser, J.C., and Schlaeppi, K. 2018. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. Microbiome 6:14.
Hartman, K., van der Heijden, M.G.A., Roussely-Provent, V., Walser, J., and Schlaeppi, K. 2017. Deciphering composition and function of the root microbiome of a legume plant. Microbiome 5:2.
Hartmann, M., Frey, B., Mayer, J., Mäder, P., and Widmer, F. 2015. Distinct soil microbial diversity under long-term organic and conventional farming. The ISME Journal 9:1177-1194.
Hawkes, C.V., Kivlin, S.N., Du, J., and Eviner, V.T. 2013. The temporal development and additivity of plant-soil feedback in perennial grasses. Plant and Soil 369:141-150.
Hendriks, M., Mommer, L., Caluwe, H., Smit-Tiekstra, A.E., van der Putten, W.H., and de Kroon, H. 2013. Independent variations of plant and soil mixtures reveal soil feedback effects on plant community overyielding. Journal of Ecology 101:287-297.
Hendriks, M., Ravenek, J. M., Smit-Tiekstra, A.E., van der Paauw, J.W., de Caluwe, H., van der Putten, W. H., de Kroon, H., and Mommer, L. 2015. Spatial heterogeneity of plant–soil feedback affects root interactions and interspecific competition. New Phytologist 207:830-840.
Hendrix, F.F., and Campbell, W.A. 1973. *Pythiums* as plant pathogens. Annual Review of Phytopathology 11:77-98.
Hetrick, B.D., Kitt, D.G., and Wilson, G.T. 1988. Mycorrhizal dependence and growth habit of warm-season and cool-season tallgrass prairie plants. Canadian Journal of Botany 66:1376-1380.
Hiruki, C. 1965. Transmission of tobacco stunt virus by *Olpidium brassicae*. Virology 25:541-549.
Hiruki, C. 1994. Multiple transmission of plant viruses by *Olpidium brassicae*. Canadian Journal of Plant Pathology 16:261-265.
Hol, W.H.G., and Van Veen, J.A. 2002. Pyrrolizidine alkaloids from *Senecio jacobaea* affect fungal growth. Journal of Chemical Ecology 28:1763-1772.
Hol, W.H.G., Vestergård, M., ten Hooven, F., Duyts, H., van de Voorde, T.F.J., and Bezemer, T.M. 2017. Transient negative biochar effects on plant growth are strongest after microbial species loss. Soil Biology and Biochemistry 115:442-451.
Holland, T.C., Bowen, P.A., Bogdanoff, C.P., Lowery, T.D., Shaposhnikova, O., Smith, S., and Hart, M.M. 2016. Evaluating the diversity of soil microbial communities in vineyards relative to adjacent native ecosystems. Applied Soil Ecology 100:91-103.
Hollister, E.B., Hu, P., Wang, A.S., Hons, F M., and Gentry, T.J. 2013. Differential impacts of brassicaceous and nonbrassicaceous oilseed meals on soil bacterial and fungal communities. FEMS Microbiology Ecology 83:632-641.
Hooks, C.R., Wang, K., Ploeg, A., and McSorley, R. 2010. Using marigold (*Tagetes* spp.) as a cover crop to protect crops from plant-parasitic nematodes. Applied Soil Ecology 46:307–320.
Huang, L., Song, L., Xia, X., Mao, W., Shi, K., Zhou, Y., and Yu, J. 2013. Plant-soil feedbacks and soil sickness: from mechanisms to application in agriculture. Journal of Chemical Ecology 39:232-242.
Huot, B., Yao, J., Montgomery, B. L., and He, S.Y. 2014. Growth–defense tradeoffs in plants: a balancing act to optimize fitness. Molecular Plant 7:1267-1287.
Innes, L., Hobbs, P.J., and Bardgett, R.D. 2004. The impacts of individual plant species on rhizosphere microbial communities in soils of different fertility. Biology and Fertility of soils 40:7-13.
Ispahani, S.K., Goud, J.C., Termorshuizen, A.J., Morton, A., and Barbara, D.J. 2008. Host specificity, but not high-temperature tolerance, is associated with recent outbreaks of *Verticillium dahliae* in chrysanthemum in the Netherlands. European Journal of Plant Pathology 122:437-442.
Jangid, K., Williams, M.A., Franzluebbers, A.J., Schmidt, T.M., Coleman, D.C., and Whitman, W.B. 2011. Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biology and Biochemistry 43:2184-2193.
Janvier, C., Villeneuve, F., Alabouvette, C., Edel-Hermann, V., Mateille, T., and Steinberg, C. 2007. Soil health through soil disease suppression: which strategy from descriptors to indicators? Soil Biology and Biochemistry 39:1-23.
Jog, R., Pandya, M., Nareshkumar, G., and Rajkumar, S. 2014. Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. Microbiology 160:778-788.

Johnson, A,W,, and Littrell, R.H. 1970. Pathogenicity of *Pythium aphanidermatum* to chrysanthemum in combined inoculations with *Belonolaimus longicaudatus* or *Meloidogyne incognita*. Journal of Nematology 2:255-259.

Johnson, N.C., and Graham, J.H. 2013. The continuum concept remains a useful framework for studying mycorrhizal functioning. Plant and Soil 363:411-419.

Jurkiewicz, A., Ryszka, P., Anielska, T., Waligórski, P., Białońska, D., Góralska, K., Tsimilli-Michael, M., and Turnau, K. 2010. Optimization of culture conditions of *Arnica montana* L.: effects of mycorrhizal fungi and competing plants. Mycorrhiza 20:293-306.

Kabir, Z., and Koide, R.T. 2000. The effect of dandelion or a cover crop on mycorrhiza inoculum potential, soil aggregation and yield of maize. Agriculture, ecosystems & environment 78:167-174.

Kageyama, K. 2014. Molecular taxonomy and its application to ecological studies of *Pythium* species. Journal of General Plant Pathology 80:314-326.

Kammerhofer, N., Egger, B., Dobrev, P., Vankova, R., Hofmann, J., Schausberger, P., and Wieczorek, K. 2015. Systemic above-and belowground cross talk: hormone-based responses triggered by *Heterodera schachtii* and shoot herbivores in *Arabidopsis thaliana*. Journal of Experimental Botany 66:7005-7017.

Kamoun, S., Furzer, O., Jones, J.D., Judelson, H.S., Ali, G.S., Dalio, R.J., et al and Cahill, D. 2015. The Top 10 oomycete pathogens in molecular plant pathology. Molecular Plant Pathology 16:413-434.

Kaplan, I., Pineda, A., and Bezemer, T.M. 2018. Application and Theory of Plant–Soil Feedbacks on Aboveground Herbivores. In Aboveground–Belowground Community Ecology (pp. 319-343). Springer, Cham.

Kardol, P., Bezemer, T.M., and van der Putten, W.H. 2006. Temporal variation in plant–soil feedback controls succession. Ecology Letters 9:1080-1088.

Kardol, P., De Deyn, G.B., Laliberte, E., Mariotte, P., and Hawkes, C.V. 2013. Biotic plant–soil feedbacks across temporal scales. Jounrla of Ecology 101:309-315.

Kelly, S., Grenyer, R., and Scotland, R.W. 2014. Phylogenetic trees do not reliably predict feature diversity. Diversity and Distributions 20:600-612.

Khan, M., and Siddiqui, Z.A. 2017. Effect of simultaneous and sequential inoculations of *Meloidogyne incognita*, *Ralstonia solanacearum* and *Phomopsis vexans* on eggplant in sand mix and fly ash mix soil. Phytoparasitica 45:599-609.

Kiernan, J.M., Hendrix, J.W., and Maronek, D.M. 1983. Fertilizer-induced pathogenicity of mycorrhizal fungi to sweetgum seedlings. Soil Biology and Biochemistry 15:257-262.

Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J. A., Franken, O., Verbruggen, E., Fellbaum, C.R., Kowalchuk, G.A., Hart, M.M., Bago, A., Palmer, T.M., West, S.A., Vandenkoornhuyse, P., Jansa, J., and Palmer, T. M. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333:880-882.

Kiers, E.T., Rousseau, R.A., West, S.A., and Denison, R.F. 2003. Host sanctions and the legume–rhizobium mutualism. Nature 425:78-81.

Kim, Y.C., Leveau, J., Gardener, B.B.M., Pierson, E.A., Pierson, L.S., and Ryu, C.M. 2011. The multifactorial basis for plant health promotion by plant-associated bacteria. Applied and Environmental Microbiology 77:1548-1555.

Kimpinski, J., Arsenault, W.J., Gallant, C.E., and Sanderson, J.B. 2000. The effect of marigolds (*Tagetes* spp.) and other cover crops on *Pratylenchus penetrans* and on following potato crops. Journal of Nematology 32:531-536.

Klein, E., Ofek, M., Katan, J., Minz, D., and Gamliel, A. 2013. Soil suppressiveness to *Fusarium* disease: shifts in root microbiome associated with reduction of pathogen root colonization. Phytopathology 103:23–33.

Kleijn, D., Bommarco, R., Fijen, T. P., Garibaldi, L. A., Potts, S. G., and van der Putten, W. H. 2018. Ecological Intensification: Bridging the Gap between Science and Practice. Trends in Ecology and Evolution 34:154-166.

Knudsen, I.M., Larsen, K.M., Jensen, D.F., and Hockenhull, J. 2002. Potential suppressiveness of different field soils to *Pythium* damping-off of sugar beet. Applied Soil Ecology 21:119-129.

Kos, S.P., Klinkhamer, P.G.L., and Leiss, K.A. 2014 Cross-resistance of chrysanthemum to western flower thrips, celery leafminer, and two-spotted spider mite. Entomologia Experimentalis et Applicata 151:198-208.

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

Kostenko, O., van de Voorde, T.F.J., Mulder, P.P.J., van der Putten, W.H., and Bezemer, T.M. 2012. Legacy effects of aboveground-belowground interactions. Ecololgy Letters 15:813-821.

Kulmatiski, A., and Beard, K.H. 2011. Long-term plant growth legacies overwhelm short-term plant growth effects on soil microbial community structure. Soil Biology and Biochemistry 43:823-830.

Kulmatiski, A., Beard, K.H., and Stark, J.M. 2006. Soil history as a primary control on plant invasion in abandoned agricultural fields. Journal of Applied Ecology 43:868-876.

Kulmatiski, A., Beard, K.H., Stevens, J.R., and Cobbold, S.M. 2008. Plant–soil feedbacks: a meta-analytical review. Ecology Letters 11:980-992.
Kuzyakov, Y., and Blagodatskaya, E. 2015. Microbial hotspots and hot moments in soil: concept & review. Soil Biology and Biochemistry 83:184-199.
Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., and Goettel, M.S. 2015. Insect pathogens as biological control agents: back to the future. Journal of Invertebrate Pathology 132:1-41.
Ladygina, N., Henry, F., Kant, M.R., Koller, R., Reidinger, S., Rodriguez, A., Saj, S., Sonnemann, I., Witt, C., and Wurst, S. 2010. Additive and interactive effects of functionally dissimilar soil organisms on a grassland plant community. Soil Biology and Biochemistry 42:2266-2275.
Ladygina, N., and Hedlund, K. 2010. Plant species influence microbial diversity and carbon allocation in the rhizosphere. Soil Biology and Biochemistry 42:162-168.
Laliberté, E., Lambers, H., Burgess, T.I., and Wright, S.J. 2015. Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. New Phytologist 206:507-521.
Lapsansky, E.R., Milroy, A.M., Andales, M.J., and Vivanco, J.M. 2016. Soil memory as a potential mechanism for encouraging sustainable plant health and productivity. Current Opinion in Biotechnology 38:137-142.
Latz, E., Eisenhauer, N., Rall, B.C., Allan, E., Roscher, C., Scheu, S., and Jousset, A. 2012. Plant diversity improves protection against soil - borne pathogens by fostering antagonistic bacterial communities. Journal of Ecology 101:597-604.
Latz, E., Eisenhauer, N., Rall, B.C., Scheu, S., and Jousset, A. 2016. Unravelling linkages between plant community composition and the pathogen-suppressive potential of soils. Scientific Reports 6:23584.
Latz, E., Eisenhauer, N., Scheu, S., and Jousset, A. 2015. Plant identity drives the expression of biocontrol factors in a rhizosphere bacterium across a plant diversity gradient. Functional Ecology 29:1225-1234.
Lay, C.Y., Hamel, C., and St-Arnaud, M. 2018. Taxonomy and pathogenicity of *Olpidium brassicae* and its allied species. Fungal Biology 122:837-846.
Leiss, K.A., Cristofori, G., van Steenis, R., Verpoorte, R., and Klinkhamer, P.G. 2013. An eco-metabolomic study of host plant resistance to Western flower thrips in cultivated, biofortified and wild carrots. Phytochemistry 93:63-70.
Leiss, K.A., Choi, Y.H., Verpoorte, R., and Klinkhamer, P.G. 2011. An overview of NMR-based metabolomics to identify secondary plant compounds involved in host plant resistance. Phytochemistry Reviews 10:205-216.
Leiss, K.A., Maltese, F., Choi, Y.H., Verpoorte, R., and Klinkhamer, P.G. 2009. Identification of chlorogenic acid as a resistance factor for thrips in chrysanthemum. Plant Physiology 150:1567-1575.
Lemanceau, P., Blouin, M., Muller, D., and Moënne-Loccoz, Y. 2017. Let the core microbiota be functional. Trends in Plant Science 22:583-595.
Lennon, J.T., and Jones, S.E. 2011. Microbial seed banks: the ecological and evolutionary implications of dormancy. Nature Reviews Microbiology 9:119-130.
Li, H., and Cheng, Z. 2015. Hoagland nutrient solution promotes the growth of cucumber seedlings under light-emitting diode light. Acta Agriculturae Scandinavica B 65:74-82.
Li, S., and Wu, F. 2018. Diversity and cooccurrence patterns of soil bacterial and fungal communities in seven intercropping systems. Frontiers in Microbiology 9:1521.
Li, J., Zheng, Y., Yan, J., Li, H., Wang, X., He, J., and Ding, G. 2013. Effects of different regeneration scenarios and fertilizer treatments on soil microbial ecology in reclaimed opencast mining areas on the Loess Plateau, China. PloS One 8:e63275.
Li, X., Wei, J., Tan, A., and Aroian, R.V. 2007. Resistance to root-knot nematode in tomato roots expressing a nematicidal *Bacillus thuringiensis* crystal protein. Plant Biotechnology Journal 5:455–464.
Liu, C., Timper, P., Ji, P., Mekete, T., and Joseph, S. 2017. Influence of Root Exudates and Soil on Attachment of *Pasteuria penetrans* to *Meloidogyne arenaria*. Journal of Nematology 49:304-310.
Liu, H., Xiong, W., Zhang, R., Hang, X., Wang, D., Li, R., and Shen, Q. 2018. Continuous application of different organic additives can suppress tomato disease by inducing the healthy rhizospheric microbiota through alterations to the bulk soil microflora. Plant and Soil 423:229-240.
Liu, Y., Zhang, N., Qiu, M., Feng, H., Vivanco, J.M., Shen, Q., and Zhang, R. 2014. Enhanced rhizosphere colonization of beneficial *Bacillus amyloliquefaciens* SQR9 by pathogen infection. FEMS Microbiology Letters 353:49-56.
Loria, R., Kers, J., and Joshi, M. 2006. Evolution of plant pathogenicity in *Streptomyces*. Annual Review of Phytopathology 44:469-487.
Lugtenberg, B., and Kamilova, F. 2009. Plant-growth-promoting rhizobacteria. Annual Review of Microbiology 63:541-556.
Lundberg, D.S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, K., del Rio, T.G., Edgar, R.C., Eickhorst, T., Ley, R.E., Hugenholtz, P., Tringe, S.G., and Edgar, R.C. 2012. Defining the core *Arabidopsis thaliana* root microbiome. Nature 488:86-90.

Lundberg, D.S., Yourstone, S., Mieczkowski, P., Jones, C.D., and Dangl, J.L. 2013. Practical innovations for high-throughput amplicon sequencing. Nature Methods 10:999-1002.
Ma, H., Pineda, A., van der Wurff, A.W., Raaijmakers, C., and Bezemer, T.M. 2017. Plant–soil feedback effects on growth, defense and susceptibility to a soil-borne disease in a cut flower crop: species and functional group effects. Frontiers in Plant Science 8:2127.
Mahato, S.B., Ganguly, A.N., and Sahu, N.P. 1982. Steroid saponins. Phytochemistry 21:959-978.
Mahmood, T., Mehnaz, S., Fleischmann, F., Ali, R., Hashmi, Z.H., and Iqbal, Z. 2014. Soil sterilization effects on root growth and formation of rhizosheaths in wheat seedlings. Pedobiologia 57:123-130.
Mallon, C.A., Le Roux, X., van Doorn, G.S., Dini-Andreote, F., Poly, F., and Salles, J.F. 2018. The impact of failure: unsuccessful bacterial invasions steer the soil microbial community away from the invader's niche. The ISME Journal 12:728-741.
Mallon, C.A., van Elsas, J.D., and Salles, J.F. 2015. Microbial invasions: the process, patterns, and mechanisms. Trends in Microbiology 23:719-729.
Maniania, N.K., Ekesi, S., Löhr, B., and Mwangi, F. 2003. Prospects for biological control of the western flower thrips, *Frankliniella occidentalis*, with the entomopathogenic fungus, *Metarhizium anisopliae*, on chrysanthemum. Mycopathologia 155:229-235.
Manners, A.G., Dembowski, B.R., and Healey, M.A. 2013. Biological control of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), in gerberas, chrysanthemums and roses. Australian Journal of Entomology 52:246-258.
Mariotte, P., Mehrabi, Z., Bezemer, T.M., De Deyn, G.B., Kulmatiski, A., Drigo, B., Ciska Veen, G.F., van der Heijden, M.G.A., and Kardol, P. 2017. Plant–soil feedback: bridging natural and agricultural sciences. Trends in Ecology and Evolution 33:129-142.
Martin, F.N., and Loper, J.E. 1999. Soilborne plant diseases caused by *Pythium* spp.: ecology, epidemiology, and prospects for biological control. Critical Reviews in Plant Sciences 18:111-181.
Mauro, R. P., Anastasi, U., Lombardo, S., Pandino, G., Pesce, R., Alessia, R., and Mauromicale, G. 2015. Cover crops for managing weeds, soil chemical fertility and nutritional status of organically grown orange orchard in Sicily. Italian Journal of Agronomy 10:101-104.
Mavrodi, O. V., Mavrodi, D. V., Parejko, J. A., Thomashow, L. S., and Weller, D. M. 2012. Irrigation differentially impacts populations of indigenous antibiotic-producing *Pseudomonas* spp. in the rhizosphere of wheat. Appllied and Environmental Microbiology 78:3214-3220.
Mazzola, M. 2002. Mechanisms of natural soil suppressiveness to soilborne diseases. Antonie van Leeuwenhoek 81:557-564.
Mazzola, M., Hewavitharana, S.S., and Strauss, S.L. 2015. *Brassica* seed meal soil amendments transform the rhizosphere microbiome and improve apple production through resistance to pathogen reinfestation. Phytopathology 105:460-469.
Mazzoleni, S., Bonanomi, G., Incerti, G., Chiusano, M.L., Termolino, P., Mingo, A., Senatore, M., Giannino, F., Cartení, F., Rietkerk, M., and Lanzotti, V. 2015. Inhibitory and toxic effects of extracellular self‐DNA in litter: a mechanism for negative plant–soil feedbacks? New Phytologies 205:1195-1210.
Meghvansi, M.K., and Varma, A. 2015. Organic Amendments and Soil Suppressiveness in Plant Disease Management. London: Springer.
Mehrabi, Z., and Tuck, S.L. 2015. Relatedness is a poor predictor of negative plant-soil feedbacks. New Phytologist 205:1071-1075.
Mehrabi, Z., Thomas, B., and Owen T.L. 2015. Plant-soil feedbacks from 30-year family-specific soil cultures: phylogeny, soil chemistry and plant life stage. Ecology and Evolution 5:2333-2339.
Mendes, L.W., Kuramae, E.E., Navarrete, A.A., van Veen, J.A., and Tsai, S.M. 2014. Taxonomical and functional microbial community selection in soybean rhizosphere. The ISME Journal 8:1577-1587.
Mendes, R., Garbeva, P., Raaijmakers, J.M. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiology Review 37:634-663.
Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H.M., Piceno, Y.M., De Santis, T.Z., Andersen, G.L., Bakker, P.A.H.M., and Raaijmakers, J.M. 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097-1100.
Mendes, L.W., Raaijmakers, J.M., de Hollander, M., Mendes, R., and Tsai, S.M. 2017. Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. The ISME Journal 12:212-224.
Mendes, R., Garbeva, P., and Raaijmakers, J.M. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiology Reviews 37:634-663.
Meyling, N.V., Lübeck, M., Buckley, E.P., Eilenberg, J., and Rehner, S.A. 2009. Community composition, host range and genetic structure of the fungal entomopathogen *Beauveria* in adjoining agricultural and seminatural habitats. Molecular Ecology 18:1282-1293.

McMurdie, P.J., and Holmes, S. 2014. Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Computational Biology 10:e1003531.
McMurtry, J.A., Moraes, G.J.D., and Sourassou, N.F. 2013. Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Systematic & Applied Acarology, 18:297-320.
Miethling, R., Wieland, G., Backhaus, H., and Tebbe, C.C. 2000. Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L33. Microbial Ecology 40:43-56.
Miglécz, T., Valkó, O., Török, P., Deák, B., Kelemen, A., Donkó, Á., Drexler, D., and Tóthmérész, B. 2015. Establishment of three cover crop mixtures in vineyards. Scientia Horticulturae 197:117-123.
Mills, K.E., and Bever, J.D. 1998. Maintenance of diversity within plant communities: soil pathogens as agents of negative feedback. Ecology 79:1595-1601.
Miransari, M. 2011. Soil microbes and plant fertilization. Applied Microbiology and Biotechnology 92:875-885.
Mommer, L., van Ruijven, J., de Caluwe, H., Smit-Tiekstra, A.E., Wagemaker, C.A.M., Joop Ouborg, N., Bö gemann, G.D., van der Weerden, G.M., Berendse, F., and de Kroon, H. 2010. Unveiling below-ground species abundance in a biodiversity experiment: a test of vertical niche differentiation among grassland species. Journal of Ecology 98:1117-1127.
Morgan, J.A.W., Bending, G.D., and White, P.J. 2005. Biological costs and benefits to plant-microbe interactions in the rhizosphere. Journal of Experimental Botany 56:1729-1739.
Morriën, E., Hannula, S.E., Snoek, L.B., Helmsing, N.R., Zweers, H., De Hollander, M., et al and Duyts, H. 2017. Soil networks become more connected and take up more carbon as nature restoration progresses. Nature Communications 8:14349.
Mouquet, N., Devictor, V., Meynard, C.N., Munoz, F., Bersier, L., Chave, J., Couteron, P., Dalecky, A., Fontaine, C., Gravel, D., et al. 2012. Ecophylogenetics: advances and perspectives. Biological Reviews 87:769-785.
Mueller, U.G., and Sachs, J.L. 2015. Engineering microbiomes to improve plant and animal health. Trends in Microbiology 23:606-617.
Münzbergová, Z., and Šurinová, M. 2015. The importance of species phylogenetic relationships and species traits for the intensity of plant-soil feedback. Ecosphere 6:1-16.
Natarajan, N., Cork, A., Boomathi, N., Pandi, R., Velavan, S., and Dhakshnamoorthy, G. 2006. Cold aqueous extracts of African marigold, *Tagetes erecta* for control tomato root knot nematode, *Meloidogyne incognita*. Crop Protection 25:1210-1213.
Nesme, J., Achouak, W., Agathos, S. N., Bailey, M., Baldrian, P., Brunel, D., et al 2016. Back to the future of soil metagenomics. Frontiers in Microbiology 7:73.
Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., and Kennedy, P.G. 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20:241-248.
Nilsson, R.H., Veldre, V., Hartmann, M., Unterseher, M., Amend, A., Bergsten, J., Kristiansson, E., Ryberg, M., Jumpponen, A., and Abarenkov, K. 2010. An open source software package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. Fungal Ecology 3:284-287.
Nouri, E., Breuillin-Sessoms, F., Feller, U., and Reinhardt, D. 2014. Phosphorus and nitrogen regulate arbuscular mycorrhizal symbiosis in Petunia hybrida. PLoS One 9:e90841.
Novara, A., Gristina, L., Guaitoli, F., Santoro, A., and Cerdà, A. 2013. Managing soil nitrate with cover crops and buffer strips in Sicilian vineyards. Solid Earth 4:255-262.
Oehl, F., Sieverding, E., Mäder, P., Dubois, D., Ineichen, K., Boller, T., and Wiemken, A. 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. Oecologia 138:574-583.
Olszewska, M. 2007. Quantitative HPLC analysis of flavonoids and chlorogenic acid in the leaves and inflorescences of *Prunus serotina* Ehrh. Acta Chromatographica 19:253-269.
Orwin, K.H., and Wardle, D.A. 2005. Plant species composition effects on belowground properties and the resistance and resilience of the soil microflora to a drying disturbance. Plant and Soil 278:205–221.
Osbourn, A.E. 2003. Saponins in cereals. Phytochemistry 62:1-4.
Packer, A., and Clay, K. 2004. Development of negative feedback during successive growth cycles of black cherry. Proceedings of the Royal Society of London. Series B: Biological Sciences 271:317-324.
Palaniyandi, S.A., Damodharan, K., Yang, S.H., and Suh, J.W. 2014. *Streptomyces* sp. strain PGPA39 alleviates salt stress and promotes growth of 'Micro Tom'tomato plants. Journal of Applied Microbiology 117:766-773.
Palaniyandi, S.A., Yang, S.H., Zhang, L., and Suh, J.W. 2013. Effects of *actinobacteria* on plant disease suppression and growth promotion. Applied Microbiology and Biotechnology 97:9621-9636.

Panke-Buisse, K., Poole, A.C., Goodrich, J.K., Ley, R.E., and Kao-Kniffin, J. 2015. Selection on soil microbiomes reveals reproducible impacts on plant function. The ISME Journal 9:980-989.

Pavoine, S., Gasc, A., Bonsall, M.B., and Mason, N.W.H. 2013. Correlations between phylogenetic and functional diversity: mathematical artefacts or true ecological and evolutionary processes? Journal of Vegetation Science 24, 781-793.

Peix, A., Ramírez-Bahena, M. H., Velázquez, E., and Bedmar, E. J. 2015. Bacterial associations with legumes. Critical Reviews in Plant Sciences 34:17-42.

Pérès, G., Cluzeau, D., Menasseri, S., Soussana, J.F., Bessler, H., Engels, C., Habekost, M., Gleixner, G., Weigelt, A., Weisser, W.W., Scheu, S., and Eisenhauer, N. 2013. Mechanisms linking plant community properties to soil aggregate stability in an experimental grassland plant diversity gradient. Plant and Soil 373:285-299.

Pérez-Jaramillo, J.E., Carrión, V.J., Bosse, M., Ferrão, L.F., de Hollander, M., Garcia, A.A., Ramírez, C.A., Mendes, R., and Raaijmakers, J.M. 2017. Linking rhizosphere microbiome composition of wild and domesticated *Phaseolus vulgaris* to genotypic and root phenotypic traits. The ISME Journal 11:2244-2257.

Pérez-Jaramillo, J.E., Carrión, V.J., de Hollander, M., and Raaijmakers, J.M. 2018. The wild side of plant microbiomes. Microbiome 6:143.

Pérez-Jaramillo, J.E., Mendes, R., and Raaijmakers, J.M. 2016. Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Molecular Biology 90:635-644.

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

Pettitt, T.R., Wainwright, M.F., Wakeham, A.J., and White, J.G. 2011. A simple detached leaf assay provides rapid and inexpensive determination of pathogenicity of *Pythium* isolates to 'all year round'(AYR) chrysanthemum roots. Plant Pathology 60:946-956.

Philippot, L., Raaijmakers, J.M., Lemanceau, P., and van Der Putten, W.H. 2013. Going back to the roots: the microbial ecology of the rhizosphere. Nature Reviews Microbiology 11:789-799.

Pieterse, C.M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., van Wees, S.C.M., and Bakker, P.A.H.M. 2014. Induced systemic resistance by beneficial microbes. Annual Review of Phytopathology 52:347-375.

Pineda, A., Kaplan, I., and Bezemer, T.M. 2017. Steering soil microbiomes to suppress aboveground insect pests. Trends in Plant Science 22:770-778.

Pineda, A., Zheng, S.J., van Loon, J.J., Pieterse, C.M., and Dicke, M. 2010. Helping plants to deal with insects: the role of beneficial soil-borne microbes. Trends in Plant Science 15:507-514.

Puławska, J., Willems, A., and Sobiczewski, P. 2012. *Rhizobium skierniewicense* sp. nov., isolated from tumours on chrysanthemum and cherry plum. International Journal of Systematic and Evolutionary Microbiology 62:895-899.

Pung, S.H., Sivasithamparam, K., Barbetti, M.J. 1991. Effect of placement and sequence of inoculation with *Fusarium oxysporum* on its interaction with *Meloidogyne arenaria* on subterranean clover. Journal of Phytopathology 132:273-286.

R Core Team 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Raaijmakers, J.M., Mazzola, M. 2016. Soil immune responses. Science 352:1392-1393.

Raaijmakers, J.M., Paulitz, T.C., Steinberg, C., Alabouvette, C., and Moënne-Loccoz, Y. 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant and Soil 321:341-361.

Reddy, P.P. 2016 Sustainable crop protection under protected cultivation. Springer, Singapore, pp 368-371.

Reidinger, S., Eschen, R., Gange, A.C., Finch, P., and Bezemer, T.M. 2012. Arbuscular mycorrhizal colonization, plant chemistry, and aboveground herbivory on *Senecio jacobaea*. Acta Oecologica 38:8-16.

Renčo, M., and Murín, J. 2013. Soil nematode assemblages in natural european peatlands of the Horná Orava protected landscape area, Slovakia. Wetlands 33:459-470.

Richardson, A.E., Barea, J.M., McNeill, A.M., and Prigent-Combaret, C. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant and Soil 321:305-339.

Ridout, M., Newcombe, G. 2016. Disease suppression in winter wheat from novel symbiosis with forest fungi. Fungal Ecology, 20:40-48.

Rottstock, T., Joshi, J., Kummer, V., and Fischer, M. 2014. Higher plant diversity promotes higher diversity of fungal pathogens, while it decreases pathogen infection per plant. Ecology 95:1907-1917.

Rudrappa, T., Czymmek, K.J., Paré, P.W., and Bais, H. P. 2008. Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiology 148:1547-1556.

Sachs, J.L., Russell, J.E., Lii, Y.E., Black, K.C., Lopez, G., and Patil, A.S. 2010. Host control over infection and proliferation of a cheater symbiont. Journal of Evolutionary Biology 23:1919-1927.

Saj, S., Mikola, J., and Ekelund, F. 2009. Species-specific effects of live roots and shoot litter on soil decomposer abundances do not forecast plant litter-nitrogen uptake. Oecologia 161:331-341.

Sánchez-Osuna, M., Barbé, J., and Erill, I. 2017. Comparative genomics of the DNA damage-inducible network in the Patescibacteria. Environmental Microbiology 19:3465-3474.

Sanguin, H., Sarniguet, A., Gazengel, K., Moënne-Loccoz, Y., and Grundmann, G.L. 2009. Rhizosphere bacterial communities associated with disease suppressiveness stages of take-all decline in wheat monoculture. New Phytologist 184:694-707.

Santhanam, R., Weinhold, A., Goldberg, J., Oh, Y., and Baldwin, I.T. 2015. Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping. Proceedings of the National Academy of Sciences 112:5013-5020.

Santoyo, G., Pacheco, C.H., Salmerón, J.H., and León, R.H. 2017. The role of abiotic factors modulating the plant-microbe-soil interactions: toward sustainable agriculture. A review. Spanish Journal of Agricultural Research 15: e03R01.

Sarma, B.K., Yadav, S.K., Singh, S., and Singh, H.B. 2015. Microbial consortium-mediated plant defense against phytopathogens: Readdressing for enhancing efficacy. Soil Biology and Biochemistry 87:25-33.

Scheublin, T.R., Ridgway, K.P., Young, J.P.W., and van der Heijden, M.G.A. 2004 Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. Appllied Environmental Microbiology 70:6240–6246.

Schisler, D.A., Slininger, P.J., and Bothast, R.J. 1997. Effects of antagonist cell concentration and two-strain mixtures on biological control of Fusarium dry rot of potatoes. Phytopathology 87:177-183.

Schlatter, D., Kinkel, L., Thomashow, L., Weller, D., and Paulitz, T. 2017. Disease suppressive soils: new insights from the soil microbiome. Phytopathology 107:1284-1297.

Shaharoona, B., Naveed, M., Arshad, M., and Zahir, Z.A. 2008. Fertilizer-dependent efficiency of *Pseudomonads* for improving growth, yield, and nutrient use efficiency of wheat (*Triticum aestivum* L.). Applied Microbiology and Biotechnology 79:147-155.

Šmilauer, P. 2001. Communities of arbuscular mycorrhizal fungi in grassland: seasonal variability and effects of environment and host plants. Folia Geobotanica 36:243-263.

Šmilauer, P., and Šmilauerová, M. 2000. Effect of AM symbiosis exclusion on grassland community composition. Folia Geobotanica 35:13-25.

Siddiqui, Z.A., Mir, R.A., and Mahmood, I. 1999. Effects of *Meloidogyne incognita*, *Fusarium oxysporum* f. sp. *pisi*, *Rhizobium* sp., and different soil types on growth, chlorophyll, and carotenoid pigments of pea. Israel Journal of Plant Sciences 47:251-256.

Siddiqui, Y., Ali, A., and Naidu, Y. 2014. Histopathological changes induced by *Meloidogyne incognita* in some ornamental plants. Crop Protection 65:216-220.

Siegel-Hertz, K., Edel-Hermann, V., Chapelle, E., Terrat, S., Raaijmakers, J.M., and Steinberg, C. 2018. Comparative microbiome analysis of a *Fusarium* wilt suppressive soil and a *Fusarium* wilt conducive soil from the Châteaurenard region. Frontiers in Microbiology 9:568.

Silva, J.C.P.D., Medeiros, F.H.V.D., and Campos, V.P. 2018. Building soil suppressiveness against plant-parasitic nematodes. Biocontrol Science and Technology 28:423-445.

Singh, M., Awasthi, A., Soni, S.K., Singh, R., Verma, R.K., and Kalra, A. 2015. Complementarity among plant growth promoting traits in rhizospheric bacterial communities promotes plant growth. Scientific Reports 5:15500.

Singh, S.L., and Pavgi, M.S. 1977. *Olpidium brassicae* in cabbage roots. Mycopathologia 62:47-52.

Sohn, B.K., Kim, K.Y., Chung, S.J., Kim, W.S., Park, S.M., Kang, J.G., Rim, Y.S., Cho, J.S., Kim, T.H., and Lee, J.H. 2003. Effect of the different timing of AMF inoculation on plant growth and flower quality of chrysanthemum. Scientia Horticulturae 98:173-183.

Soler, R., van der Putten, W.H., Harvey, J.A., Vet, L.E.M., Dicke, M., and Bezemer, T.M. 2012. Root herbivore effects on aboveground multitrophic interactions: patterns, processes and mechanisms. Journal of Chemical Ecology 38:755-767.

Song, A., Zhao, S., Chen, S., Jiang, J., Chen, S., Li, H., Chen, Y., Chen, X., Fang, W., and Chen, F. 2013. The abundance and diversity of soil fungi in continuously monocropped chrysanthemum. Scientific World Journal 2013:632920.

Soto, M.J., Domínguez-Ferreras, A., Pérez-Mendoza, D., Sanjuán, J., and Olivares, J. 2009. Mutualism versus pathogenesis: the give-and-take in plant–bacteria interactions. Cellular Microbiology 11:381-388.

Soto, M.J., Sanjuan, J., and Olivares, J. 2006. Rhizobia and plant-pathogenic bacteria: common infection weapons. Microbiology. 152:3167-3174.

St-Denis, A., Kneeshaw, D., Bélanger, N., Simard, S., Laforest-Lapointe, I., and Messier, C. 2017. Species-specific responses to forest soil inoculum in planted trees in an abandoned agricultural field. Applied Soil Ecology 112:1-10.

Stevens, C.J., Wilson, J., and McAllister, H.A. 2012. Biological flora of the British Isles: *Campanula rotundifolia*. Journal of Ecology 100:821-839.

Stiles, C.M., Datnoff, L.E., and Rayside, P.A. 2007. *Pythium* spp. isolated from bermudagrass during overseed transitions in Florida and pathogenicity of *Pythium irregulare* on *Poa trivialis*. Plant Disease 91:1237-1244.

Sturz, A.V., and Kimpinski, J. 2004. Endoroot bacteria derived from marigolds (*Tagetes* spp.) can decrease soil population densities of root-lesion nematodes in the potato root zone. Plant and Soil 262:241-249.

Subrahmanyam, P., and McDonald, D. 1980. Occurrence of *Olpidium brassicae* on groundnut. Transactions of the British Mycological Society 75:506-509.

Swenson, W., Wilson, D.S., and Elias, R. 2000. Artificial ecosystem selection. Proceedings of the National Academy of Sciences 97:9110-9114.

Szoboszlay, M., Lambers, J., Chappell, J., Kupper, J.V., Moe, L.A., and McNear Jr, D.H. 2015. Comparison of root system architecture and rhizosphere microbial communities of Balsas teosinte and domesticated corn cultivars. Soil Biology and Biochemistry 80:34-44.

Taghavi, S., Garafola, C., Monchy, S., Newman, L., Hoffman, A., Weyens, N., Barac, T., Vangronsveld, J., and van der Lelie, D. 2009. Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. Applied and Environmental Microbiology 75:748-757.

Tamm, L., Thürig, B., Bruns, C., Fuchs, J.G., Köpke, U., Laustela, M., Leifert, C., Mahlberg, N., Nietlispach, B., Schmidt, C., Weber, F., and 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.

Teakle, D.S., and Hiruki, C. 1964. Vector specificity in *Olpidium*. Virology 24:539-544.

Tedersoo, L., Anslan, S., Bahram, M., Põlme, S., Riit, T., Liiv, I., Kõljalg, U., Kisand, V., Nilsson, R.H., Hildebrand, F., Bork, P., and Abarenkov, K. 2015. Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. MycoKeys 10:1-43.

Teixeira, L.C., Peixoto, R.S., Cury, J.C., Sul, W.J., Pellizari, V.H., Tiedje, J., and Rosado, A.S. 2010. Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. The ISME Journal 4:989-1001.

Thomsen, C.N., and Hart, M.M. 2018. Using invasion theory to predict the fate of arbuscular mycorrhizal fungal inoculants. Biological Invasions 20:1-12.

Thuerig, B., Fließbach, A., Berger, N., Fuchs, J.G., Kraus, N., Mahlberg, N., Nietlispach, B., and Tamm, L. 2009 Re-establishment of suppressiveness to soil-and air-borne diseases by re-inoculation of soil microbial communities. Soil Biology and Biochemistry 41:2153-2161.

Tian, L., and Shi, W. 2014. Short-term effects of plant litter on the dynamics, amount, and stoichiometry of soil enzyme activity in agroecosystems. European Journal of Soil Biology 65:23-29.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., and Siemann, E. 1997. The influence of functional diversity and composition on ecosystem processes. Science 277:1300-1302.

Toju, H., Peay, K.G., Yamamichi, M., Narisawa, K., Hiruma, K., Naito, K., Fukuda, S., Ushio, M., Nakaoka, S., Onoda, Y., Yoshida, K., Schlaeppi, K., Bai, Y., Sugiura, R., Ichihashi, Y., Minamisawa, K., and Kiers, E.T. 2018. Core microbiomes for sustainable agroecosystems. Nature Plants 4:247-257.

Torres-Cortés, G., Millán, V., Fernández-González, A.J., Aguirre-Garrido, J.F., Ramírez-Saad, H.C., Fernández-López, M., Toro, N., and Martínez-Abarca, F. 2012. Bacterial community in the rhizosphere of the cactus species *Mammillaria carnea* during dry and rainy seasons assessed by deep sequencing. Plant and Soil 357:275-288.

Turner, M.F., Heuberger, A.L., Kirkwood, J.S., Collins, C.C., Wolfrum, E.J., Broeckling, C.D., Prenni, J.E., and Jahn, C.E. 2016. Non-targeted metabolomics in diverse sorghum breeding lines indicates primary and secondary metabolite profiles are associated with plant biomass accumulation and photosynthesis. Frontiers in Plant Science 7:953.

Vale, M., Nguyen, C., Dambrine, E., and Dupouey, J.L. 2005. Microbial activity in the rhizosphere soil of six herbaceous species cultivated in a greenhouse is correlated with shoot biomass and root C concentrations. Soil Biology and Biochemistry 37:2329-2333.

van Bruggen, A.H.C., and Semenov, A.M. 2000. In search of biological indicators for soil health and disease suppression. Applied Soil Ecology 15:13-24.

van Dam, N.M. 2009. Belowground herbivory and plant defenses. Annual Review of Ecology, Evolution, and Systematics 40:373-391.

van de Mortel, J.E., de Vos, R.C.H., Dekkers, E., Pineda, A., Guillod, L., Bouwmeester, K., van Loon, J.J.A., Dicke, M., and Raaijmakers, J.M. 2012. Metabolic and transcriptomic changes induced in *Arabidopsis* by the rhizobacterium *Pseudomonas fluorescens* SS101. Plant Physiology 160:2173-2188.

van de Voorde, T.F., van der Putten, W.H., and Bezemer, T.M. 2011. Intra-and interspecific plant–soil interactions, soil legacies and priority effects during old-field succession. Journal of Ecology 99:945-953.
van der Bom, F., Nunes, I., Raymond, N.S., Hansen, V., Bonnichsen, L., Magid, J., Nybroe, O., and Jensen, L.S. 2018. Long-term fertilisation form, level and duration affect the diversity, structure and functioning of soil microbial communities in the field. Soil Biology and Biochemistry 122:91-103.
van der Gaag, D.J., and Wever, G. 2005. Conduciveness of different soilless growing media to *Pythium* root and crown rot of cucumber under nearcommercial conditions. European Journal of Plant Pathology 112:31–41.
van der Heijden, M.G.A., and Hartmann, M. 2016. Networking in the plant microbiome. PLoS Biology 14:e1002378.
van der Hoeven, A.P. 1986. Chrysanthemum production in the Netherlands. Working-party on Greenhouse Chrysanthemum 197:11-20.
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., and 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., Bradford, M.A., Brinkman, P.E., van der Voorde, T. F., and Veen, G.F. 2016. Where, when and how plant–soil feedback matters in a changing world. Functional Ecology 30:1109-1121.
van der Wurff, A.W.G., Streminska, M.A., Corsten, R., and Sloten, M. 2014. Biostimulators, agents and disease suppression of *Pythium* in Chrysanthemum: Indicators for disease suppression in the soil (No. 1314). Wageningen UR Greenhouse Horticulture.
van der Wurff, A.W.G., van Slooten, M.A., Hamelink, R., Böhne, S., van Wensveen, W. 2010. Soil suppressiveness towards *Meloidogyne*, *Verticillium* or *Pythium* in greenhouse horticulture. ISHS Acta Horticulture 915:141-149.
van Elsas, J.D., Chiurazzi, M., Mallon, C.A., Elhottovā, D., Krištůfek, V., and Salles, J.F. 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. Proceedings of the National Academy of Sciences 109:1159-1164.
van Loon, L.C. 2007. Plant responses to plant growth-promoting rhizobacteria. In New Perspectives and Approaches in Plant Growth-Promoting Rhizobacteria Research (pp. 243-254). Springer, Dordrecht.
van Os, G.J., and van Ginkel, J.H. 2001. Suppression of *Pythium* root rot in bulbous *Iris* in relation to biomass and activity of the soil microflora. Soil Biology and Biochemistry 33:1447-1454.
Vanterpool, T.C. 1930. *Asterocystis radicis* in the roots of cereals in Saskatchewan. Phytopathology 20:677-680.
Veiga, R.S., Faccio, A., Genre, A., Pieterse, C.M., Bonfante, P., and van der Heijden, M.G.A. 2013. Arbuscular mycorrhizal fungi reduce growth and infect roots of the non-host plant *Arabidopsis thaliana*. Plant, Cell & Environment 36:1926-1937.
Veresoglou, S.D., Sen, R., Mamolos, A.P., and Veresoglou, D.S. 2011. Plant species identity and arbuscular mycorrhizal status modulate potential nitrification rates in nitrogen-limited grassland soils. Journal of Ecology 99:1339-1349.
Viaene, T., Langendries, S., Beirinckx, S., Maes, M., and Goormachtig, S. 2016. *Streptomyces* as a plant's best friend? FEMS Microbiology Ecology 92:1-10.
Vrain, T., DeYoung, R., Hall, J., and Freyman, S. 1996. Cover crops resistant to root-lesion nematodes in raspberry. HortScience 31:1195-1198.
Vukicevich, E., Lowery, T., Bowen, P., Úrbez-Torres, J.R., and Hart, M. 2016. Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. Agronomy for Sustainable Development 36:48.
Wagner, M. R., Lundberg, D. S., Tijana, G., Tringe, S. G., Dangl, J. L., and Mitchell-Olds, T. 2016. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. Nature Communications 7:12151.
Wang, B., Teng, Y., Xu, Y., Chen, W., Ren, W., Li, Y., Christie, P. and Luo, Y. 2018. Effect of mixed soil microbiomes on pyrene removal and the response of the soil microorganisms. Science of The Total Environment 640:9-17.
Wang, G.Z., Li, H.G., Christie, P., Zhang, F.S., Zhang, J.L., and Bever, J.D. 2017. Plant-soil feedback contributes to intercropping overyielding by reducing the negative effect of take-all on wheat and compensating the growth of faba bean. Plant and Soil 415:1-12.
Wang, J., Li, X., Xing, S., Ma, Z., Hu, S., and Tu, C. 2017. Bio-organic fertilizer promotes plant growth and yield and improves soil microbial community in continuous monoculture system of *Chrysanthemum morifolium* cv. Chuju. International Journal of Agriculture and Biology 19:563-568.
Wang, M., Bezemer, T.M., van der Putten, W.H., and Biere, A. 2015. Effects of the timing of herbivory on plant defense induction and insect performance in ribwort plantain (*Plantago lanceolata* L.) depend on plant mycorrhizal status. Journal of Chemical Ecology 41:1006-1017.

Ward Jr, J.H. 1963. Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association 58:236-244.
Wardecki, T., Brötz, E., De Ford, C., von Loewenich, F.D., Rebets, Y., Tokovenko, B., Luzhetskyy, A., and Merfort, I. 2015. Endophytic *Streptomyces* in the traditional medicinal plant *Arnica montana* L.: secondary metabolites and biological activity. Antonie van Leeuwenhoek 108:391-402.
Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., and Wall, D.H. 2004. Ecological linkages between aboveground and belowground biota. Science 304:1629-1633.
Wardle, D.A., Bonner, K.I., Barker, G.M., Yeates, G.W., Nicholson, K.S., Bardgett, R.D., Watson, R.N., and Ghani, A. 1999. Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. Ecological Monographs 69:535-568.
Wardle, D.A., Yeates, G.W., Williamson, W., and Bonner, K.I. 2003. The response of a three trophic level soil food web to the identity and diversity of plant species and functional groups. Oikos 102:45-56.
Warembourg, F.R., Roumet, C., and Lafont, F. 2003. Differences in rhizosphere carbon-partitioning among plant species of different families. Plant and Soil 256:347-357.
Webb, C.O., Ackerly, D.D., and Kembel, S.W. 2008. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. Bioinformatics 24:2098-2100.
Webb, C.O., and Donoghue, M.J. 2005. Phylomatic: tree assembly for applied phylogenetics. Molecular Ecology Notes 5:181-183.
Webb, C.O., Gilbert, G.S., and Donoghue, M.J. 2006. Phylodiversity-dependent seedling mortality, size structure, and disease in a Bornean rain forest. Ecology 87:123-131.
Wei, Z., Yang, T., Friman, V. P., Xu, Y., Shen, Q., and Jousset, A. 2015. Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. Nature Communications 6:8413.
Weigelt, A., King, R., Bol, R., and Bardgett, R.D. 2003. Inter-specific variability in organic nitrogen uptake of three temperate grassland species. Journal of Plant Nutrition and Soil Science 166:606-611.
Weiner, J. 2017. Applying plant ecological knowledge to increase agricultural sustainability. Journal of Ecology 105:865-870.
Weller, D.M., Raaijmakers, J.M., Gardener, B.B.M., and Thomashow, L.S. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annual Review of Phytopathology 40:309-348.
Werner, G.D., and Kiers, E.T. 2015. Order of arrival structures arbuscular mycorrhizal colonization of plants. New Phytologist 205:1515-1524.
Whitelaw-Weckert, M.A., Rahman, L., Appleby, L.M., Hall, A., Clark, A.C., Waite, H., Hardie, W.J. 2013 Co-infection by Botryosphaeriaceae and *Ilyonectria* spp. fungi during propagation causes decline of young grafted grapevines. Plant Pathology 62:1226-1237.
Wieland, G., Neumann, R., and Backhaus, H. 2001. Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. Applied and Environmental Microbiology 67:5849-5854.
Wikström, N., Savolainen, V., and Chase, M.W. 2001. Evolution of the angiosperms: calibrating the family tree. Proceedings of the Royal Society B 268:2211-2220.
Witt, C., and Setälä, H. 2010. Do plant species of different resource qualities form dissimilar energy channels below-ground? Applied Soil Ecology 44:270-278.
Wu, H., Raza, W., Fan, J., Sun, Y., Bao, W., Liu, D., Huang, Q., Mao, Z., Shen, Q., and Miao, W. 2008. Antibiotic effect of exogenously applied salicylic acid on in vitro soilborne pathogen, *Fusarium oxysporum* f. sp. niveum. Chemosphere 74:45-50.
Wubs, E.J., and Bezemer, T.M. 2016. Effects of spatial plant–soil feedback heterogeneity on plant performance in monocultures. Journal of Ecology 104:364-376.
Wubs, E.J., and Bezemer, T.M. 2018. Temporal carry-over effects in sequential plant–soil feedbacks. Oikos 127:220-229.
Wubs, E.J., Melchers, P.D., and Bezemer, T.M. 2018. Potential for synergy in soil inoculation for nature restoration by mixing inocula from different successional stages. Plant and Soil 1-10.
Wubs, E.J., van der Putten, W.H., Bosch, M., and Bezemer, T.M. 2016. Soil inoculation steers restoration of terrestrial ecosystems. Nature Plants 2:16107.
Wurst, S., and Ohgushi, T. 2015. Do plant-and soil-mediated legacy effects impact future biotic interactions? Functional Ecology 29:1373-1382.
Wurst, S., and van Beersum, S. 2009. The impact of soil organism composition and activated carbon on grass-legume competition. Plant and Soil 314:1-9.
Xiong, W., Li, R., Ren, Y., Liu, C., Zhao, Q., Wu, H., Jousset, A., and Shen, Q. 2017. Distinct roles for soil fungal and bacterial communities associated with the suppression of vanilla *Fusarium* wilt disease. Soil Biology and Biochemistry 107:198-207.

Xue, W., Huang, L., Yu, F.H., and Bezemer, T.M. 2018. Intraspecific aggregation and soil heterogeneity: competitive interactions of two clonal plants with contrasting spatial architecture. Plant and Soil 425:231-240.

Yu, D., Sinkkonen, A., Hui, N., Kurola, J.M., Kukkonen, S., Parikka, P., Vestberg, M., and Romantschuk, M. 2015. Molecular profile of microbiota of Finnish commercial compost suppressive against *Pythium* disease on cucumber plants. Applied Soil Ecology 92:47-53.

Yuan, Z., Druzhinina, I.S., Labbé, J., Redman, R., Qin, Y., Rodriguez, R., Zhang, C., Tuskan, G.A., and Lin, F. 2016. Specialized microbiome of a halophyte and its role in helping non-host plants to withstand salinity. Scientific Reports 6:32467.

Zabihi, H.R., Savaghebi, G.R., Khavazi, K., Ganjali, A., and Miransari, M. 2011. *Pseudomonas* bacteria and phosphorous fertilization, affecting wheat (*Triticum aestivum* L.) yield and P uptake under greenhouse and field conditions. Acta Physiologiae Plantarum 33:145-152.

Zachow, C., Müller, H., Tilcher, R., and Berg, G. 2014. Differences between the rhizosphere microbiome of *Beta vulgaris* ssp. *maritima*—ancestor of all beet crops—and modern sugar beets. Frontiers in Microbiology, 5:415.

Zhang, N., van der Putten, W.H., and Veen, G.F. 2016. Effects of root decomposition on plant-soil feedback of early- and mid-successional plant species. New Phytologist 212:220-231.

Zhang, Y., Wang, L., Yao, Y., Yan, J., and He, Z. 2012. Phenolic acid profiles of Chinese wheat cultivars. Journal of Cereal Science 56:629-635.

Zhao, J., Zeng, Z., He, X., Chen, H., and Wang, K. 2015. Effects of monoculture and mixed culture of grass and legume forage species on soil microbial community structure under different levels of nitrogen fertilization. European Journal of Soil Biology 68:61-68.

Zhou, K., Zhou, X., Yang, L., Hao, F., Wang, Z., and Guo, W. 2009. Autotoxic effects of Chrysanthemum (*Chrysanthemum morifolium* (Ramat) Tzvel.). Allelopathy Journal 24:91-102.

Zhou, X., Liu, J., and Wu, F. 2017. Soil microbial communities in cucumber monoculture and rotation systems and their feedback effects on cucumber seedling growth. Plant and Soil 415:507-520.

Summary

Mono-cropping often leads to the rapid build-up of pathogens in the soil, and this can cause great losses in agriculture yields. Steaming of soil is a commonly done in commercial chrysanthemum greenhouses to eliminate soil pathogens, but at the same time this also eliminates beneficial microbes. Besides consumption of gas and manpower, this is an unsustainable practice, because it leaves an empty niche in the soil where pathogens can easily re-establish. Soil from natural ecosystems harbors great diversity and potentially more beneficial soil microbes than agricultural soils. Plants can selectively recruit beneficial microbes, sanction non-efficient microbes, and hence can amplify the beneficial effects of soil microbial communities. Thus, by growing wild plant species in their native soil we may create functional microbiomes that could be used to improve the growth and health of crops. The aim of my thesis was to test the feasibility of using grassland soil and native plant species to increase chrysanthemum growth and health through plant-soil feedback principles; and hence whether soil and plant species from natural ecosystems can be used in agricultural systems to increase yield.

I first screened the effects of 37 plant species that belong to three functional groups (grasses, forbs, legumes), on chrysanthemum growth, health and the concentration of leaf chlorogenic acid. The chrysanthemum plants were also exposed or not to the oomycete pathogen *Pythium ultimum*. The experiment had two phases, in the conditioning phase, the wild plant species were grown individually in soil from a grassland to create 37 inocula. In the test phase, chrysanthemum cuttings were grown in soil consisting of 10% conditioned inocula mixed with 90% sterilized soil, with or without exposure to *Pythium*. I found that conditioning plant species had strong species-specific and functional group-specific effects on chrysanthemum via the soil, but their effects were not related with their phylogenetic distance to chrysanthemum. In general, grass-conditioned soil inocula had more positive effects on chrysanthemum growth and health than forb-conditioned inocula and legume-conditioned inocula. Chrysanthemum grown in soil with inocula conditioned by wild plant species did not have significantly higher biomass than in 100% sterilized soil. However, in presence of *Pythium*, inoculation with five out of nine grass-conditioned soils resulted in significantly higher chrysanthemum biomass than in 100% sterilized soil. The concentration of leaf chlorogenic acid was positively correlated with chrysanthemum shoot biomass. These results support the idea that plant species and soil from natural ecosystems can be used to improve the growth and health of chrysanthemum.

Then, I tested how homogenously mixing two plant-conditioned inocula affects the growth and health of chrysanthemum. Based on the results from the previous experiment, I selected eight plant species with positive or negative effects on chrysanthemum. These plant species were grown in the grassland soil individually to create eight inocula. Then, I created mixtures of inocula by mixing pairs of the plant-conditioned inocula (including mixing each inoculum with itself) in a 1:1 ratio. Chrysanthemum cuttings

were grown in soil consisting of 10% mixed inocula with 90% sterilized soil. For each mixed inoculum, I calculated the predicted effect based on the sum of the effects of the component soils, and compared this with the observed effects. I found on average, that mixing two plant-conditioned soil led to lower chrysanthemum biomass than predicted, but better chrysanthemum health than predicted. Moreover, the larger the differences between the effects of two plant-conditioned inocula were, the more likely the observed effect of the mixture was higher than the predicted effect. The results of this experiment indicate that potential increased soil microbial diversity caused by soil mixing is positive for chrysanthemum health with a cost in terms of growth. However, the antagonistic interactions between two soil inocula on chrysanthemum growth could be improved by mixing two inocula that have very distinctive effects on chrysanthemum growth.

In a next experiment, I tested the carry-over effects of the same eight plant species on chrysanthemum growth and health during two successive growth cycles with exposure to different soil-borne diseases in each cycle. In the first growth cycle, chrysanthemum was grown in sterilized soil inoculated with eight plant-conditioned inocula and 100% sterilized soil (un-inoculated soil), and either exposed to the oomycete pathogen *Pythium ultimum*, to the plant parasitic nematode *Meloidogyne incognita* or not exposed (control). In the second growth cycle, new chrysanthemum cuttings were grown again in the soil from the previous round or in new 100% sterilized soil as control, either exposed to *Pythium ultimum*, to a diseased soil inoculum (collected from a commercial chrysanthemum greenhouse with soil disease problems), or without a disease treatment. I found that at the end of the second growth cycle, plant-conditioned inocula that were added in the beginning of the cultivation were still influencing chrysanthemum performance. Further, chrysanthemum had strong negative conspecific feedback effects, and in general, in all soils in which chrysanthemum had been grown for one cycle we observed lower chrysanthemum biomass than in new sterilized soil. However, inoculation with plant-conditioned inocula significantly reduced the negative conspecific effects of chrysanthemum. The *Pythium* or *Meloidogyne* treatments did not significantly influence chrysanthemum growth. An interesting finding is that inoculation with diseased soil led to significant negative effects on chrysanthemum biomass when added to new sterilized soil, but on the contrary, it resulted in increased chrysanthemum growth in soils in which chrysanthemum had been grown before. With certain soil inocula (*i.e. Lolium perenne* conditioned soil), chrysanthemum biomass in pots where diseased soil was added was even higher than in pots with new sterilized soil and without a disease treatment. The results of this experiment indicate that the effects of inoculation with plant-conditioned soil on chrysanthemum may last for more than one growth cycle. When comparing with the effects of un-inoculated soil (sterilized soil), the effects of plant-conditioned inocula became more obvious after a second growth cycle, when the plants were also exposed to diseases.

Finally, I tested the effects of inoculation of soil from eight wild plant species on the chrysanthemum root microbiome, on plant growth, and on the susceptibility to an aboveground insect (thrips) using soil collected from a commercial chrysanthemum greenhouse. In the conditioning phase, I grew eight plant species either in the greenhouse soil or in natural grassland soil. In the test phase, the conditioned soil inocula were then added to 90% sterilized greenhouse soil or 90% live greenhouse soil. I found that chrysanthemum performance was worse in live greenhouse soil than in sterilized greenhouse soil, and inoculation with either plant-conditioned soil or with greenhouse soil in which first wild plant species had been grown did not significantly influence this negative effect. However, inoculation of greenhouse soil that was conditioned by *Lolium perenne* or *Festuca filiformis* led to higher bacterial diversity and lower relative abundance of pathogenic fungi in chrysanthemum roots. The best soil combination for chrysanthemum growth was conditioned grassland soil inoculated into sterilized greenhouse soil. In this combination soils had a more connected and complex root-associated microbial network than soils from the other three soil combinations. Soil sterilization sustained the highest chrysanthemum growth, however, roots of chrysanthemum plants grown in 100% sterilized soil also had a higher relative abundance of pathogenic fungi than plants grown in soils with plant-conditioned inocula. Roots of chrysanthemum plants grown in live greenhouse soil were dominated by *Olpidium brassicae*. OTUs from *Streptomyces* spp were also abundant in roots and one OTU was highly positively and another OTU was negatively correlated with chrysanthemum growth, suggesting that they may be important for chrysanthemum growth. Overall, soil inoculation or changes in the root microbiome were not related to the susceptibility of chrysanthemum to thrips. The results of this experiment indicate that reversing the negative effect of greenhouse soil on chrysanthemum is difficult, especially with an inoculation ratio of 1:9. However, inoculating with natural soil in which wild plant species had been grown first leads to a more functional and potentially more beneficial microbiome for chrysanthemum.

In conclusion, my studies demonstrate that plant species and soil from natural ecosystems can be used to improve the growth and health of chrysanthemum through plant-soil feedback principles. Compared with 100% sterilized soil, inoculation with plant-conditioned soil does not significantly increase chrysanthemum growth in absence of other diseases. However, with exposure to pathogens, inoculation with plant-conditioned soil can significantly increase chrysanthemum growth. Moreover, inoculation with plant-conditioned soil significantly reduced the negative conspecific soil effects of chrysanthemum. My studies highlight the importance of plant and soil from natural ecosystems in enhancing the sustainability of horticulture. Future studies should focus on disentangling the microbiome in the inoculated soil and understanding its functions.

Samenvatting

Mono-cropping leidt vaak tot de snelle opbouw van ziekteverwekkers in de bodem en dit kan grote verliezen in landbouwopbrengsten veroorzaken. In commerciële chrysanthemumkassen wordt de grond regelmatig gestoomd om bodempathogenen te elimineren, maar tegelijkertijd worden dan ook nuttige microben geëlimineerd. Naast het verbruik van gas en mankracht is dit een niet-duurzame praktijk, omdat het een vacuum in de bodem achterlaat waardoor ziekteverwekkers gemakkelijk kunnen hervestigen. De bodem van natuurlijke ecosystemen herbergt vaak een grotere diversiteit en bevat vaak meer potentieel nuttige bodemmicroben dan landbouwgronden. Planten kunnen selectief nuttige microben aantrekken, niet-efficiënte micro-organismen sanctioneren en daardoor de gunstige effecten van microbiële gemeenschappen in de bodem versterken. Door wilde plantensoorten te kweken in hun natuurlijke grond, kunnen we functionele microbiomen maken die kunnen worden gebruikt om de groei en gezondheid van gewassen te verbeteren. Het doel van mijn proefschrift is om de haalbaarheid te testen van het gebruik van grasland en inheemse plantensoorten om de groei en gezondheid van het gewas chrysant te verhogen via principes van plant-bodem terugkoppelingen; en dus of bodem- en plantensoorten uit natuurlijke ecosystemen kunnen worden gebruikt in landbouwsystemen om de opbrengst te verhogen.

Ik onderzocht eerst de effecten via de bodem van 37 plantensoorten die behoren tot drie functionele groepen (grassen, kruiden en stikstofbinders) op de groei van chrysant, en op de gezondheid en de concentratie van chlorogenic zuur in de bladeren. De chrysant planten werden ook blootgesteld of niet aan de ziekteverwekkende schimmel *Pythium ultimum*. Het experiment had twee fasen, in de conditioneringsfase werden de wilde plantensoorten individueel gekweekt in bodem verzameld uit een natuurlijk grasland om 37 inocula te creeren. In de testfase werden chrysantstekken gegroeid in grond bestaande uit 10% geconditioneerd inoculum gemengd met 90% gesteriliseerde grond, met of zonder blootstelling aan *Pythium*. Ik ontdekte dat de conditionerende plantensoorten sterke soort-specifieke en functionele groep-specifieke effecten hadden op chrysant via de grond, maar dat hun effecten niet waren gerelateerd aan de fylogenetische afstand van de plantesoort tot chrysant. In het algemeen hadden inocula van grassen meer positieve effecten op de groei en gezondheid van chrysant dan inocula van kruiden of van stikstofbinders. Chrysanten die groeiden in geinoculeerde grond hadden geen significant hogere biomassa dan in 100% gesteriliseerde grond. In aanwezigheid van *Pythium* resulteerde inenting met vijf van de negen geconditioneerde bodems door grassoorten in aanzienlijk hogere biomassa van chrysant dan in 100% gesteriliseerde grond. De concentratie van chlorogeenzuur in het blad was positief gecorreleerd met chrysanthemum shoot biomassa. Deze resultaten ondersteunen het idee dat plantensoorten en de bodem van natuurlijke ecosystemen kunnen worden gebruikt om de groei en gezondheid van chrysanthemum te verbeteren.

Vervolgens heb ik getest hoe het homogeen mengen van twee inocula de groei en gezondheid van chrysant beïnvloedt. Op basis van de resultaten van het vorige experiment selecteerde ik acht plantensoorten met positieve of negatieve effecten op chrysant. Deze plantensoorten werden afzonderlijk in grond verzameld uit een natuurlijk grasland gekweekt om acht inocula te creëren. Vervolgens heb ik mengsels van twee inocula gemaakt door (inclusief het mengen van elk inoculum met zichzelf) in een verhouding van 1: 1. Chrysantenstekken werden geplant in grond bestaande uit 10% van het gemengde inoculum met 90% gesteriliseerde grond. Voor elk gemengd inoculum, berekende ik het voorspelde effect op basis van de som van de effecten van de twee afzonderlijke inocula, en vergeleek dit met de waargenomen effecten. Gemiddeld vond ik dat het mengen van twee inocula leidde tot lagere biomassa van chrysant dan voorspeld, maar een betere gezondheid dan was voorspeld. Bovendien, hoe groter de verschillen tussen de effecten van twee aparte inocula op de planten waren, hoe groter de kans was dat het waargenomen effect van het mengsel hoger was dan het voorspelde effect. De resultaten van dit experiment tonen aan dat de potentiële verhoogde bodemmicrobiële diversiteit veroorzaakt door het mengen van twee inocula positief is voor de gezondheid van chrysant maar met een kostenpost voor de groei. De antagonistische interacties tussen twee inocula op de groei van chrysanten kunnen echter worden verbeterd door twee inocula te mengen met een zeer verschillende werking op de groei van chrysanten.

In een volgend experiment testte ik de langere termijneffecten van inoculeren met grond van dezelfde acht plantensoorten op de groei en gezondheid van chrysanten gedurende twee opeenvolgende groeicycli, met blootstelling aan verschillende door de bodem overgedragen ziekten in elke cyclus. In de eerste groeicyclus werd de chrysanten gekweekt in gesteriliseerde grond geënt met een van de acht inocula of in 100% gesteriliseerde grond (niet-geënte grond) en ofwel blootgesteld aan de ziekteverwekker *Pythium ultimum*, aan de plantparasitaire nematode *Meloidogyne incognita* of niet blootgesteld (controle). In de tweede groeicyclus werden nieuwe chrysantstekken opnieuw gegroeid in de bodem van de vorige ronde of in nieuwe 100% gesteriliseerde bodem als controle, en weer blootgesteld aan *Pythium ultimum*, of aan grond verzameld uit een commerciële chrysantekas met daarin bodemziekten, of gekweekt zonder een ziektebehandeling. Ik ontdekte dat aan het einde van de tweede groeicyclus het inoculeren aan het begin van de teelt, nog steeds de prestaties van de chrysant beïnvloedde. Verder vertoonde chrysant sterke negatieve conspecifieke terugkoppellingen via de bodem. In alle bodems waarin chrysant gedurende één cyclus had gegroeid was de biomassa lager dan in nieuwe gesteriliseerde grond. Inoculeren met bodem waarin eerst andere soorten hadden gegroeid verminderde echter deze negatieve effecten. De *Pythium*- of *Meloidogyne*-behandelingen hadden geen significante invloed op de groei van chrysant. Een interessante bevinding is dat inenting met zieke bodem verzameld uit de kas, leidde tot aanzienlijke negatieve effecten op de biomassa van chrysant ald dit was toevoegd aan nieuwe gesteriliseerde grond. Echter, het resulteerde in verhoogde groei in bodems waarin eerder chrysant had

gegroeid. Met bepaalde bodeminocula (dat wil zeggen, met grond waarin eerst *Lolium perenne* had gegroeid) was de biomassa van chrysant in potten waar ook zieke grond werd toegevoegd zelfs hoger dan in potten met nieuwe gesteriliseerde grond en zonder een ziektebehandeling. De resultaten van dit experiment tonen aan dat de effecten van inoculatie met geconditioneerde grond op chrysanthemum meer dan één groeicyclus kunnen voortbestaan. Bij vergelijking met de effecten van niet-geënte grond (gesteriliseerde grond) werden de effecten van de inocula duidelijker na een tweede groeicyclus, voor planten die ook aan ziekten werden blootgesteld.

Ten slotte testte ik de effecten van het enten van bodem waar eerst acht wilde plantensoorten in gegroeid hebben op het microbioom van de chrysanthemum, alsmede op de plantengroei en de gevoeligheid voor een bovengronds insect (trips). In de conditioneringsfase groeiden de acht plantensoorten in de grond verzameld uit een commerciele chrysantekas of in natuurlijke graslandgrond. In de testfase werden de geconditioneerde bodeminocula vervolgens toegevoegd aan 90% gesteriliseerde of 90% levende kasgrond. Ik vond dat chrysant slechter groeide in levende dan in gesteriliseerde kasgrond, en dat aanenten met ofwel geconditioneerde graslandgrond of kasgrond waarin eerst wilde planten gegroeid hadden, dit effect niet signficant verminderde. De inoculatie met grond waarin *Lolium perenne* of *Festuca filiformis* gegroeid had leidde echter tot een hogere bacteriële diversiteit en een lagere relatieve abundantie van pathogene schimmels in chrysantenwortels. Planten groeiden het beste in gesterriliseerde kasgrond aangeent met geconditioneerde graslandgrond. In deze combinatie was er een meer verbonden en complex root-geassocieerd microbieel netwerk dan in bodems van de andere drie grondcombinaties. Bodemsterilisatie leidde tot de hoogste biomassa van chrysant, maar wortels van chrysantenplanten die in 100% gesteriliseerde grond waren gekweekt, hadden ook een hogere relatieve abundantie van pathogene schimmels dan wortels van planten die in geinoculeerde bodems groeiden. De wortels van chrysantenplanten gekweekt in levende kasgrond werden gedomineerd door *Olpidium brassica*e. OTU's van *Streptomyces* spp waren ook overvloedig aanwezig in wortels. Eén van die OTUs was zeer positief en een andere negatief gecorreleerd met groei van de plant. Over het algemeen waren bodeminoculatie of veranderingen in het wortelmicrobioom niet gerelateerd aan de gevoeligheid van chrysant voor trips. De resultaten van dit experiment tonen aan dat het afwentelen van het negatieve effect dat aanwezig is in kasgrond op chrysant moeilijk is, zeker met een inoculatieverhouding van 1: 9. Het inoculeren met natuurlijke grond waarin eerst wilde plantensoorten hebben gegroeid, leidt echter tot een functioneler en mogelijk gunstiger microbioom voor chrysant.

Mijn studies tonen aan dat plantensoorten en de bodem van natuurlijke ecosystemen kunnen worden gebruikt om de groei en gezondheid van chrysant te verbeteren via principes van plant-bodem terugkoppelingen. In vergelijking met 100% gesteriliseerde grond verhoogt inoculatie met geconditioneerde grond de groei van chrysant niet significant in afwezigheid van andere ziekten. Bij

blootstelling aan ziekteverwekkers kan aanenten met geconditioneerde grond de groei van chrysanten echter aanzienlijk verhogen. Bovendien verminderde aanenten met door planten geconditioneerde grond significant de negatieve conspecifieke bodemeffecten van chrysant. Mijn studies benadrukken de belangrijke rol die wilde planten en de natuurlijke bodems kunnen spelen bij het verbeteren van de duurzaamheid van de tuinbouw. Toekomstige studies moeten gericht zijn op het ontwarren van het microbioom in aangeënte bodems en het begrijpen van de functies ervan.

Curriculum vitae

Haikun Ma was born on 17th March 1991 in Xinyang, Henan Province, China. She received her bachelor degree at Hunan Agricultural University, China in 2011. During her bachelor study, she focused on the construction of phylogenetic tree of ruminants based on the milk protein gene sequences. In 2011, she started her master study at Nankai University, China. She worked on the profile of soil microbial community in different ecosystems in China. She finished her thesis and received her master degree in 2014. In 2014, she received a grant from 'China Scholarship Council' for supporting her PhD study abroad. In the same year, she started her PhD project at Terrestrial Ecology department of Netherlands Institute of Ecology (NIOO-KNAW), under the supervision of Prof.T. Martijn Bezemer, Dr. Andre van der Wurff and Dr. Ana Pineda. She focused on using plant-soil feedback principles to improve the growth and health of a horticultural crop. The findings of her PhD researches are described in this thesis.

Publications

Ma, H., Pineda, A., van der Wurff, A.W., Bezemer, T.M. (2018). Carry-over effects of soil inoculation on plant growth and health under sequential exposure to soil-borne diseases. Plant and Soil, 433, 257-270.

Ma, H., Pineda, A., van der Wurff, A.W., Bezemer, T.M. (2018). Synergistic and antagonistic effects of mixing monospecific soils on plant-soil feedbacks. Plant and Soil, 429, 271-279.

Ma, H., Pineda, A., van der Wurff, A.W., Raaijmakers, C., Bezemer, T.M. (2017). Plant-soil feedback effects on growth, defense and susceptibility to a soil-borne disease in cut flower crop: species and functional group effects. Frontiers in plant science, 8, 2127.

Ma, H., Bai, G., Sun, Y., Kostenko, O., Zhu, X., Lin, S., Ruan, W., Zhao, N., Bezemer, T.M. (2016). Opposing effects of nitrogen and water addition on soil bacterial and fungal communities in the Inner Mongolia steppe: a field experiment. Applied soil ecology, 108, 128-135.

Bai, G., Xu, H., Fu, Y., Wang, X., Shen, G., **Ma, H.**, Feng, X., Pan, J., Gu, X., Guo, Y., Shapiro-Ilan, DI.,Ruan, W. (2016). A Comparison of Novel Entomopathogenic Nematode Application Methods for Control of the Chive Gnat, Bradysia odoriphaga (Diptera: Sciaridae). Journal of economic entomology, 109, 1-8.