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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 *Actinomyces* 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 strains 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 identify 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 until 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 thuringiensis*, 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, thrips 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.

Chapter 1

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

Chapter 1

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.