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

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# 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;<br>No leaf yellowness | **n.s.** or > | No significant increase in yield or leaf yellowness |
| *Pythium ultimum* | Yield decreased;<br>No effects on leaf yellowness | < | Yield increased;<br>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;<br>Leaf yellowness increased | < | Yield decreased;<br>Leaf yellowness increased |
| Control - Diseased soil inoculum | Yield increased;<br>Leaf yellowness decreased | < | Yield increased;<br>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;<br>Leaf yellowness decreased | **n.s.** | Yield increased;<br>Leaf yellowness decreased |
| *Pythium* - *Pythium* | Yield increased;<br>No significant effects on leaf yellowness | **n.s.** | No effects on yield;<br>No significant effects on leaf yellowness |
| *Meloidogyne* - Control | No significant effects | **n.s.** | No significant effects |
| *Meloidogyne* - Diseased soil inoculum | Yield increased;<br>Leaf yellowness decreased | < | Yield increased;<br>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).

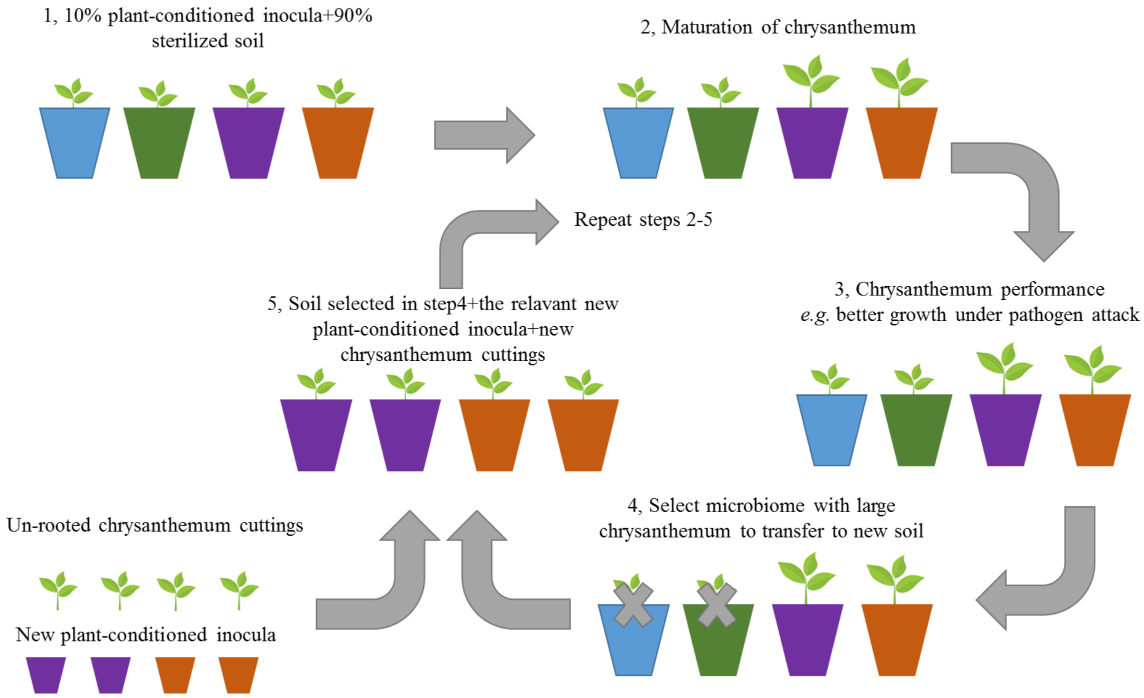

**Fig. 6.1.** Selection on microbiomes that produce best chrysanthemum performance. Chrysanthemums are inoculated with 10% inocula conditioned by different wild plant species + 90% sterilized soil (Step 1). The host-microbiome associations are allowed to mature (Step2), pathogen treatments can be added 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.