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

Ma, H.

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

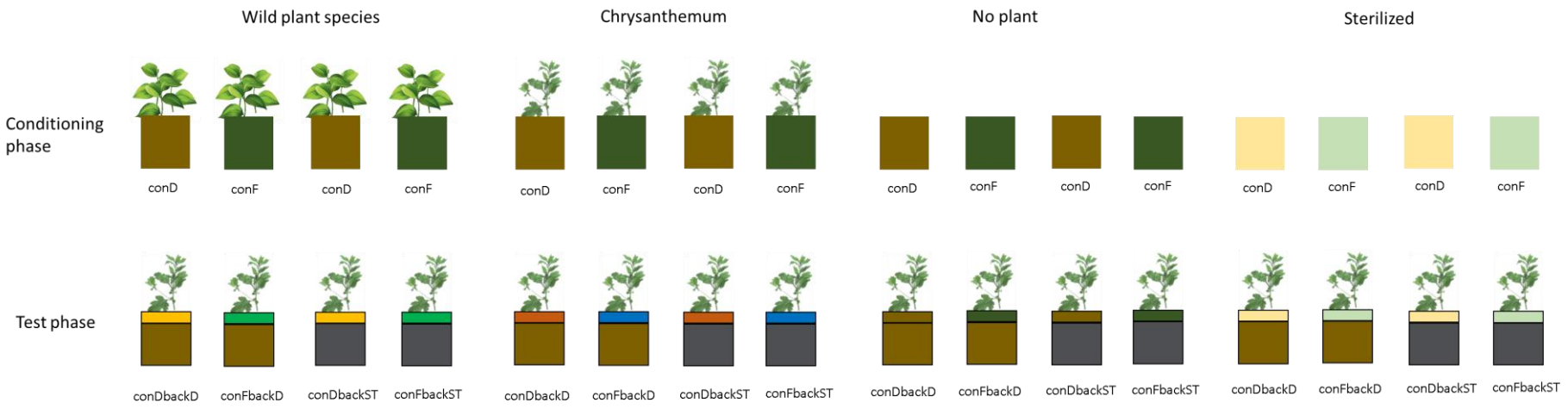


Fig.5.1 Experimental design. For clarity, only one wild plant species out of the eight tested is shown. Details about the conditioning plant species are described in the Materials and Methods section. In the conditioning phase, dark green soil indicates soil collected from a natural grassland; brown 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

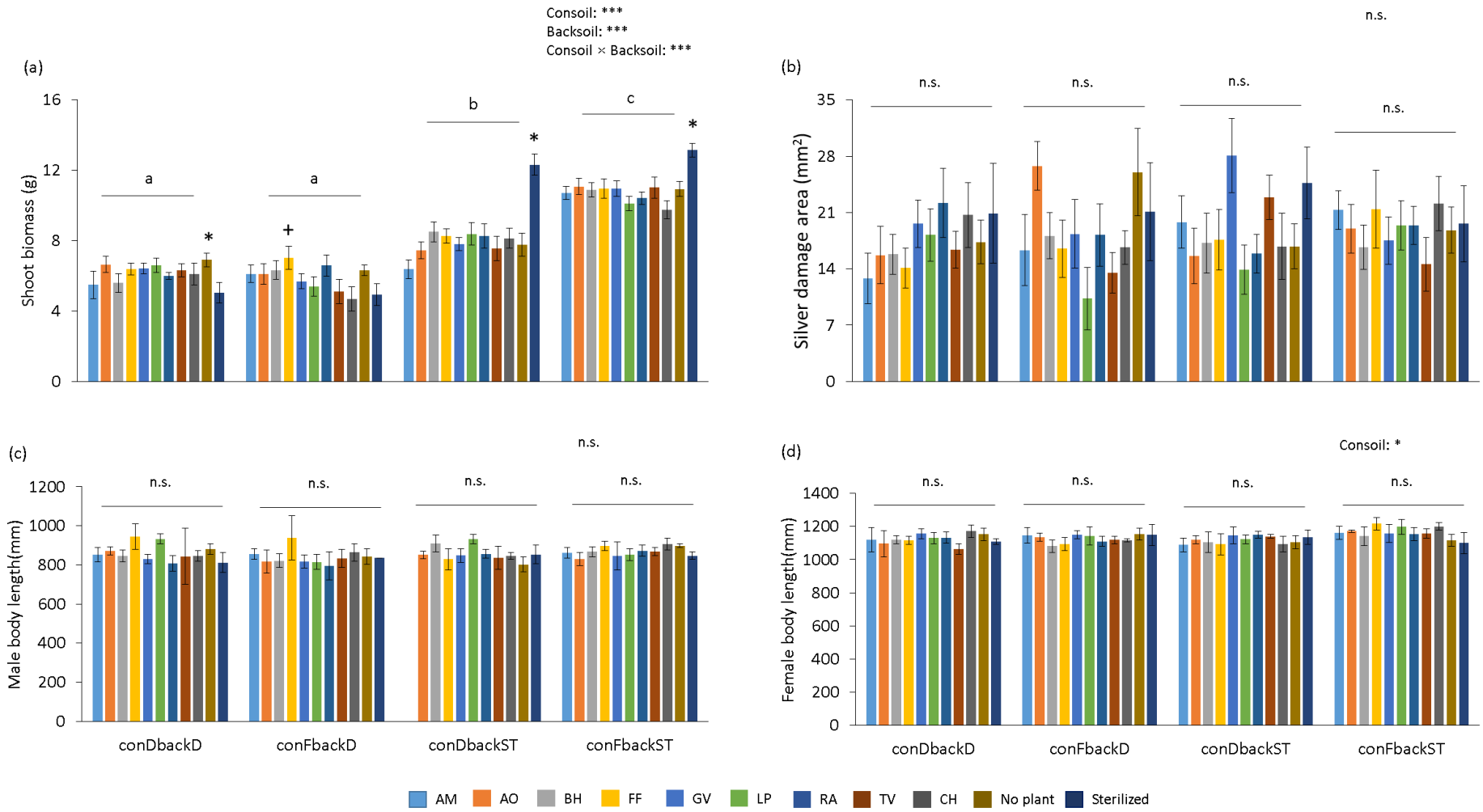


Fig.5.2 Chrysanthemum shoot biomass (a), leaf silver damage area (b), body length of male thrips (c) and body length of female thrips (d) in different conditioning and background soil type combinations conditioned by wild plant species, chrysanthemum, no-plant conditioning and sterilized no-plant conditioning 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

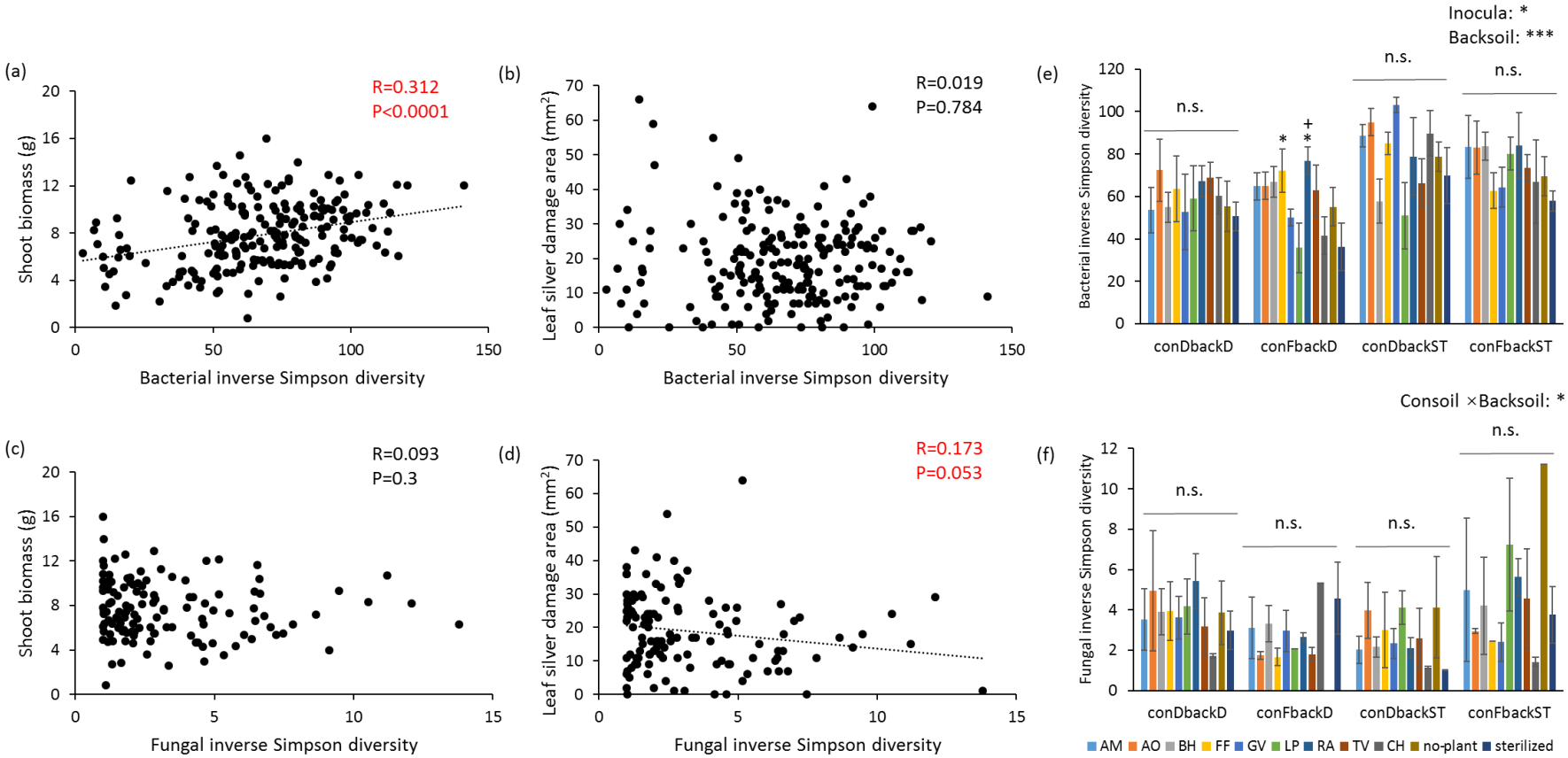


Fig.5.3 Relationships between root-associated bacterial and fungal diversity with chrysanthemum shoot biomass (a,c), leaf silver damage area (b,d) and bacterial and fungal diversity in different soil treatments (e,f). In each bar plot, statistics of the overall effects are presented in the upper part 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.

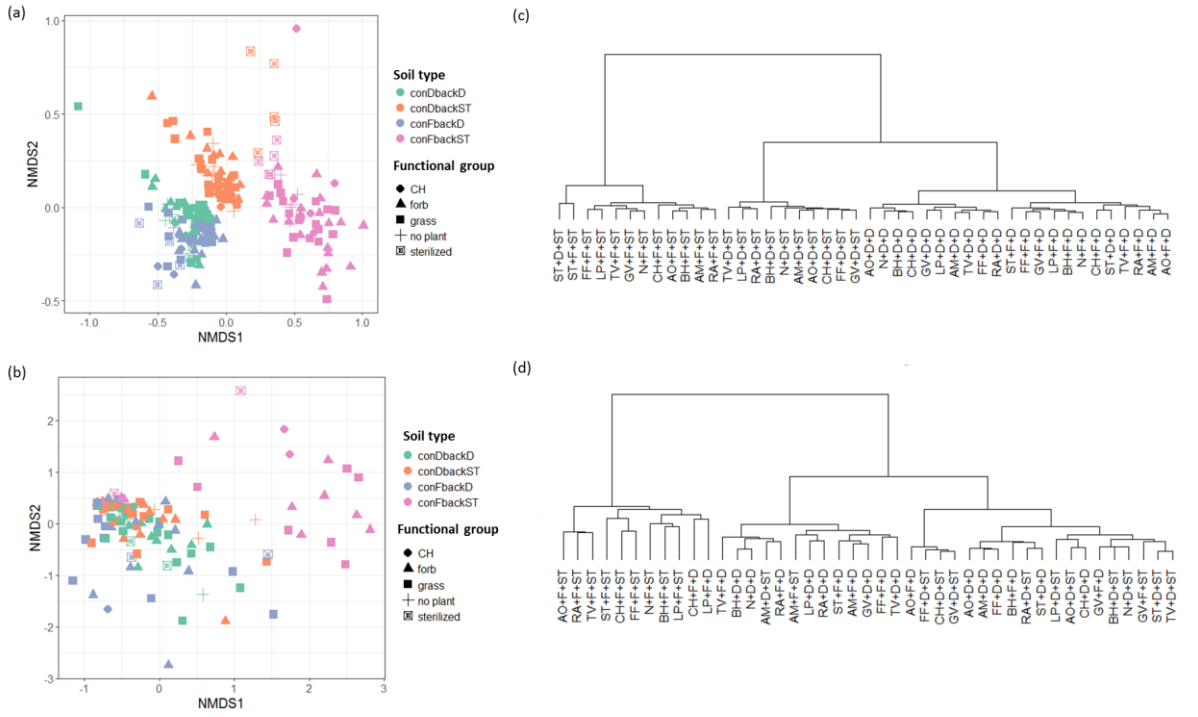


Fig.5.4 Non-metric multidimensional scaling (NMDS) plot performed on taxonomic profile (OTU level for 16s and ITS DNA) of root-associated bacteria (a) and fungi (b), and the hierarchical cluster analysis of bray-curtis similarities between each treatment on root-associated bacteria (c) and fungi (d). 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.

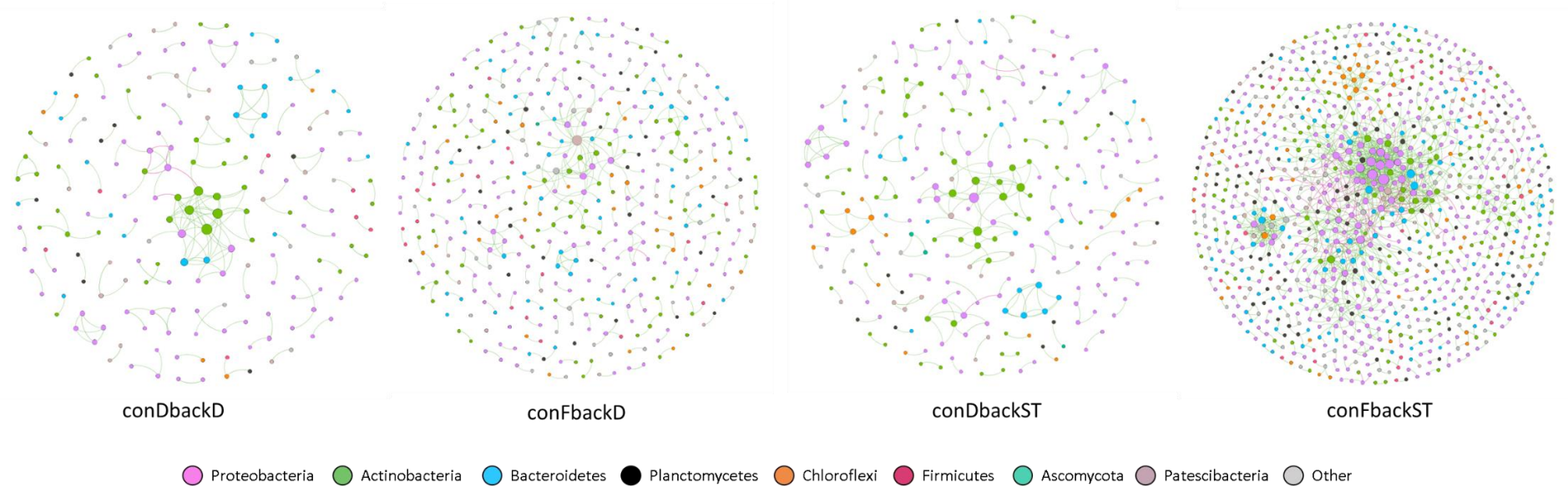


Fig.5.5 Network co-occurrence analysis of chrysanthemum root-associated microbial communities in the four types of conditioning and background soil combinations. A connection stands for a Spearman Rank correlation with magnitude > 0.7 (both positive and negative) that is statistically significant ($P < 0.05$ 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).

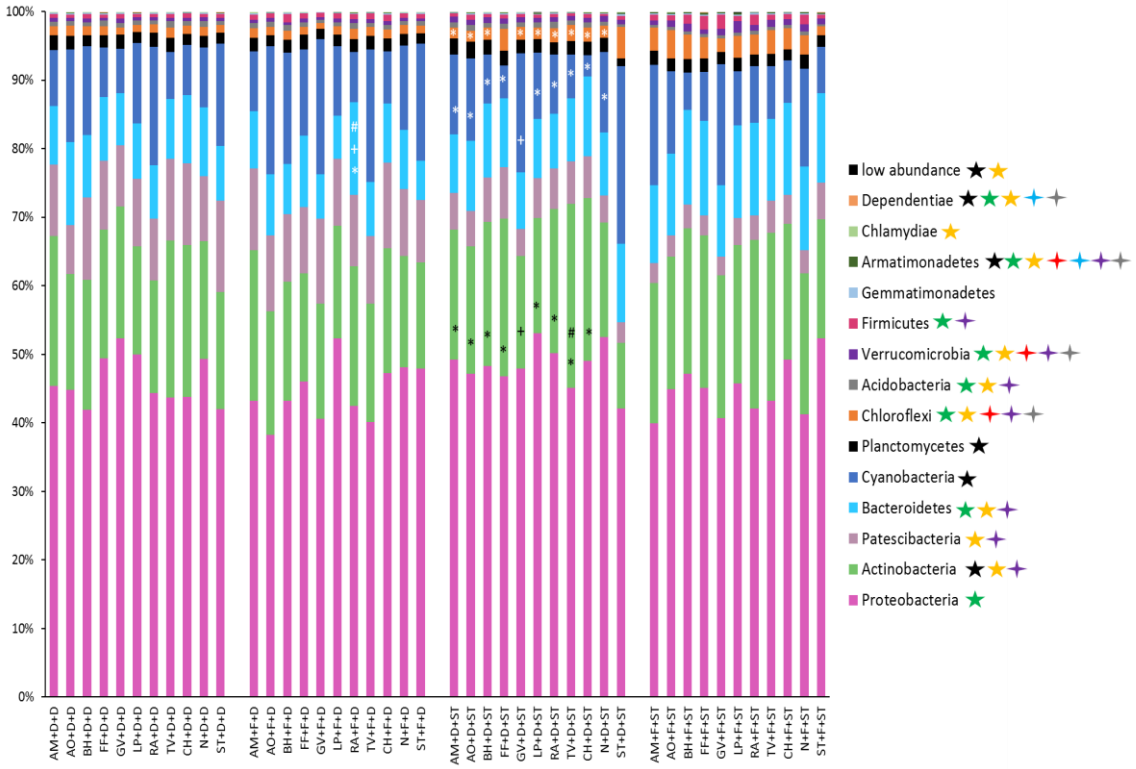
Roots of chrysanthemum growing in greenhouse soil inocula and sterilized background soil that were conditioned by *Lolium perenne*, *Anthoxanthum odoratum* and *Achillea millefolium* had lower relative abundance of *Olpidiomycota* are higher relative abundance of *Ascomycota* (except for *Achillea millefolium*) 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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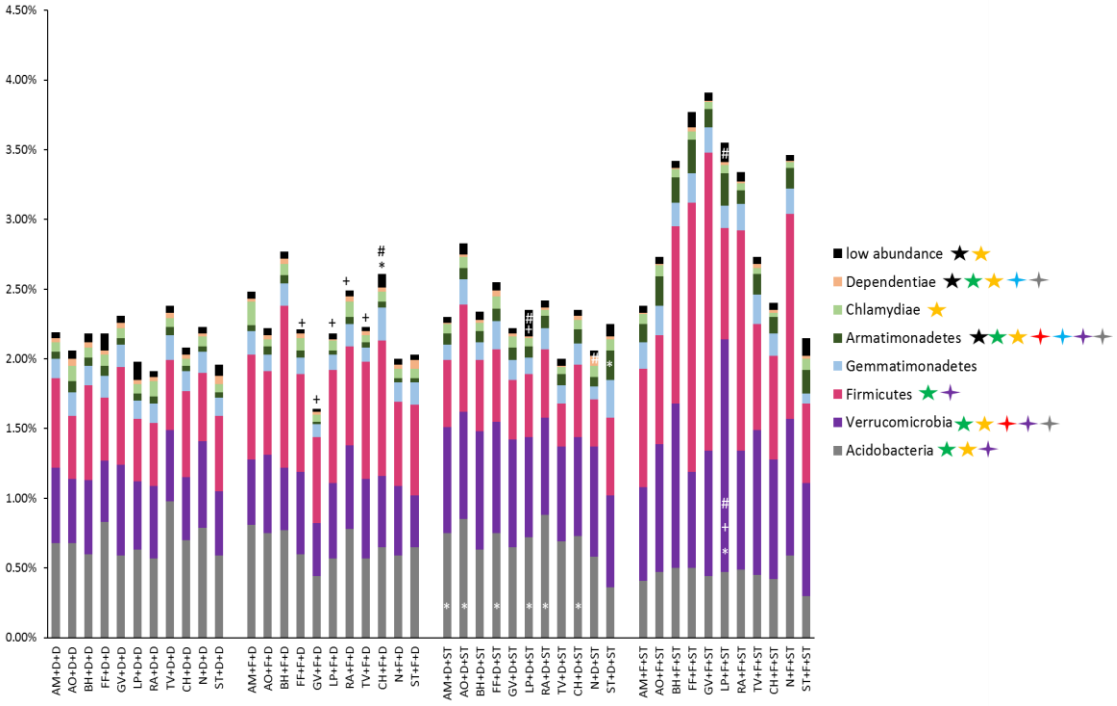
Chlamydiae
Armatimonadetes
Gemmatimonadetes
Firmicutes
Verrucomicrobia
Acidobacteria
Chloroflexi
Planctomycetes
Cyanobacteria
Bacteroidetes
Patescibacteria
Actinobacteria
Proteobacteria

(b)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Armatimonadetes
Gemmatimonadetes
Firmicutes
Verrucomicrobia
Acidobacteria

(c)

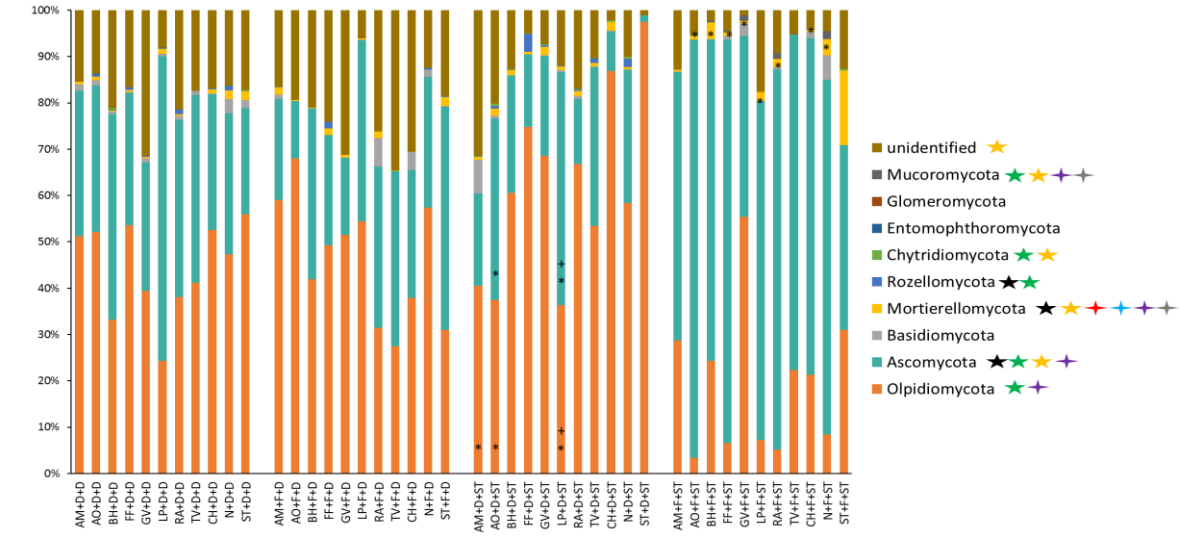

Fig.5.6 The relative abundance of bacterial phyla (a,b) and fungal phyla (c) in each soil treatment. Fig.5.6a and b both show bacterial phyla composition, Fig.5.6b shows the relative low abundance phyla which are not visible in Fig.5.6a. Five-point stars following the legend of each phylum represent 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

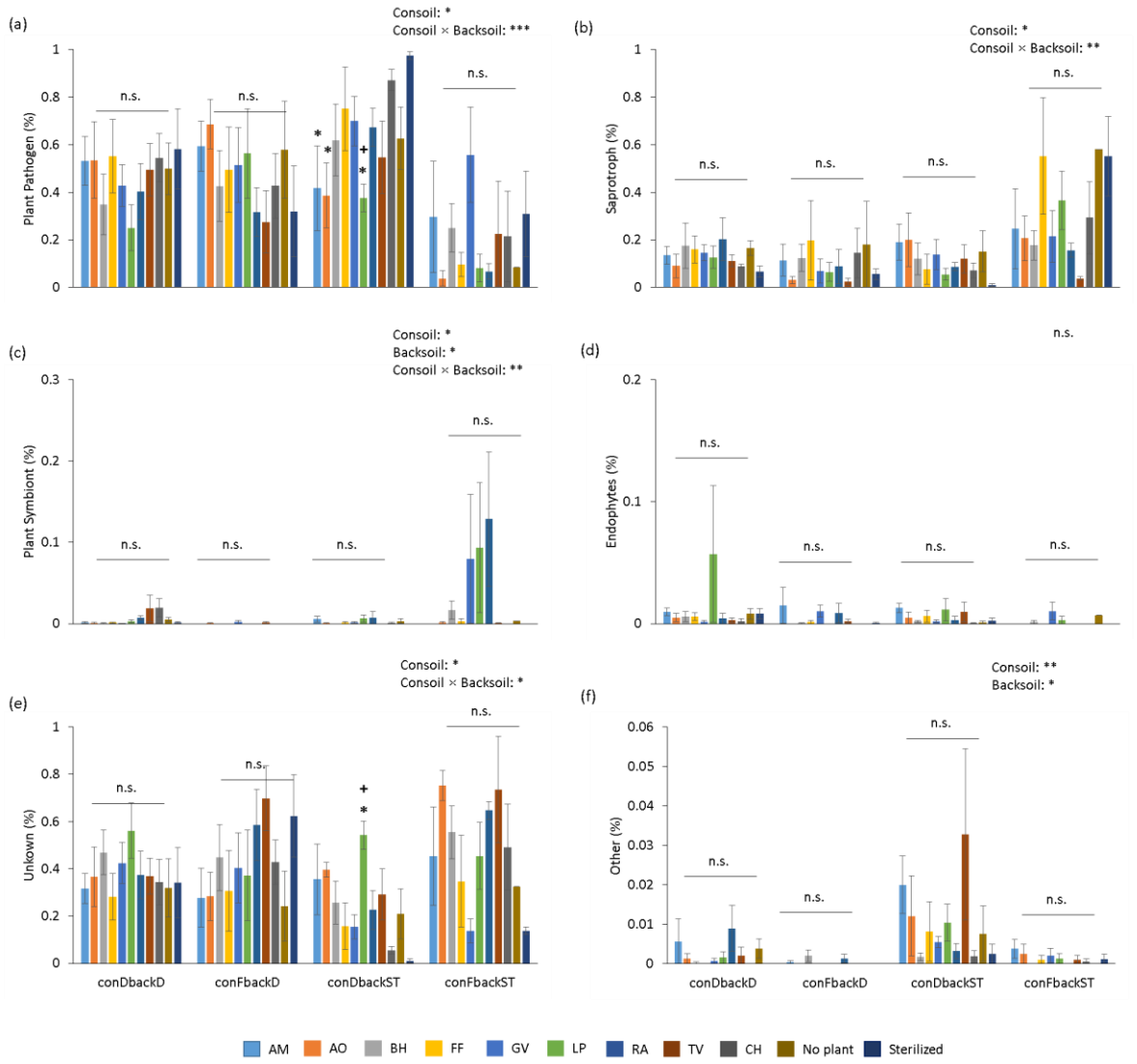

Fig.5.7 The relative abundance of plant pathogenic fungi (a), saprotophic fungi (b), plant symbiontic fungi (c), endophytic fungi (d), fungi with unkown functions (e) and other functional group fungi (d) in different soil treatments. Other functional groups include fungi that are marine species, nematode 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

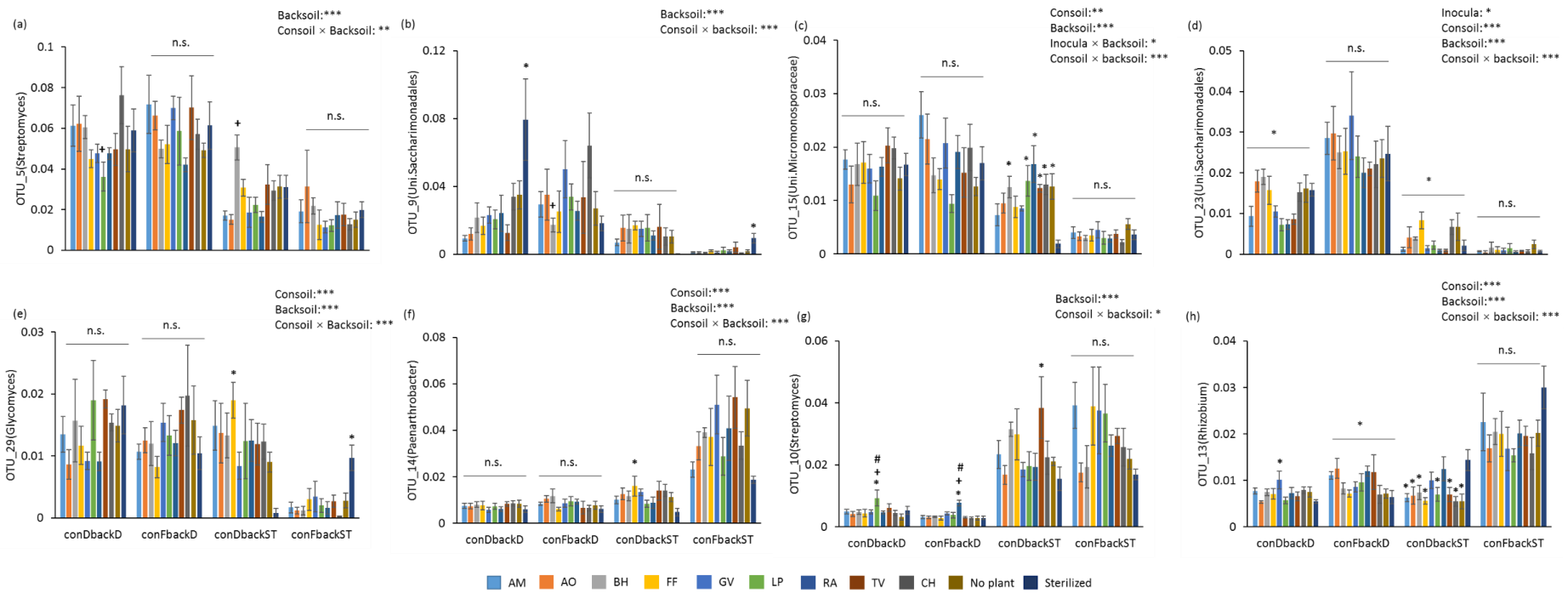

Fig.5.8 The relative abundance of OTUs in different soil treatments. The selection of the eight OTUs is from Table S5.3 that represents OTUs that are highly correlated with plant shoot biomass, and had an average relative abundance across all samples of more than 1%. The correlation between these OTUs 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.

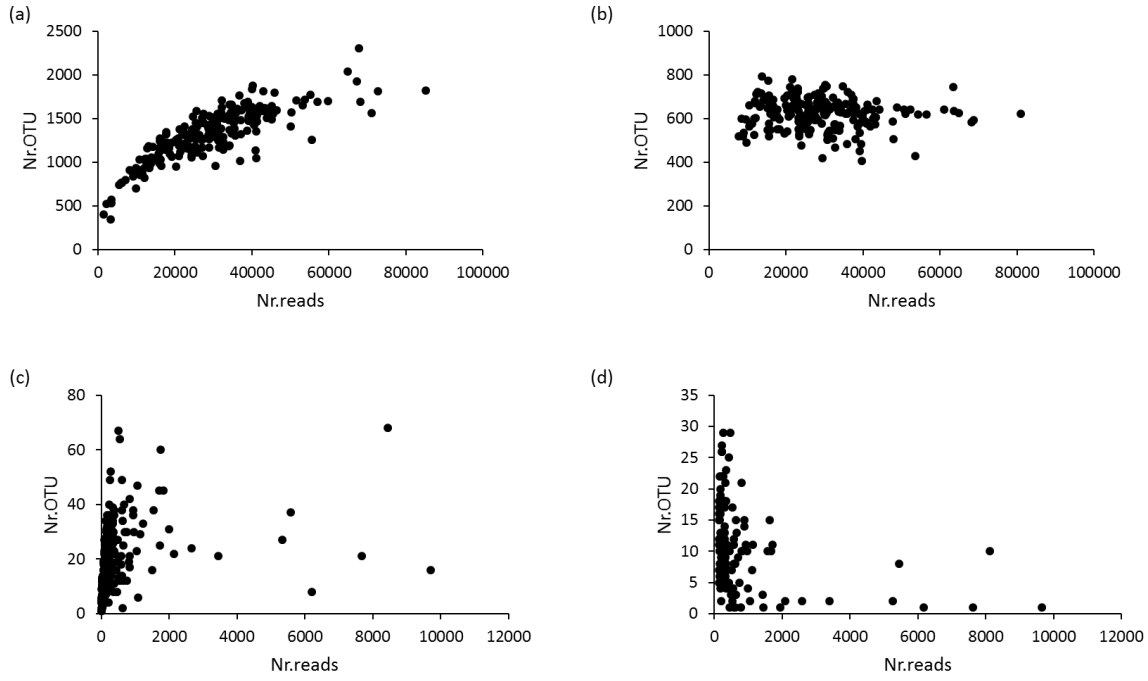

Fig.S5.2 Correlations between bacterial diversity and fungal diversity to body length of female and male thrips. "n.s." indicates no significant correlation was found using Pearson correlation.

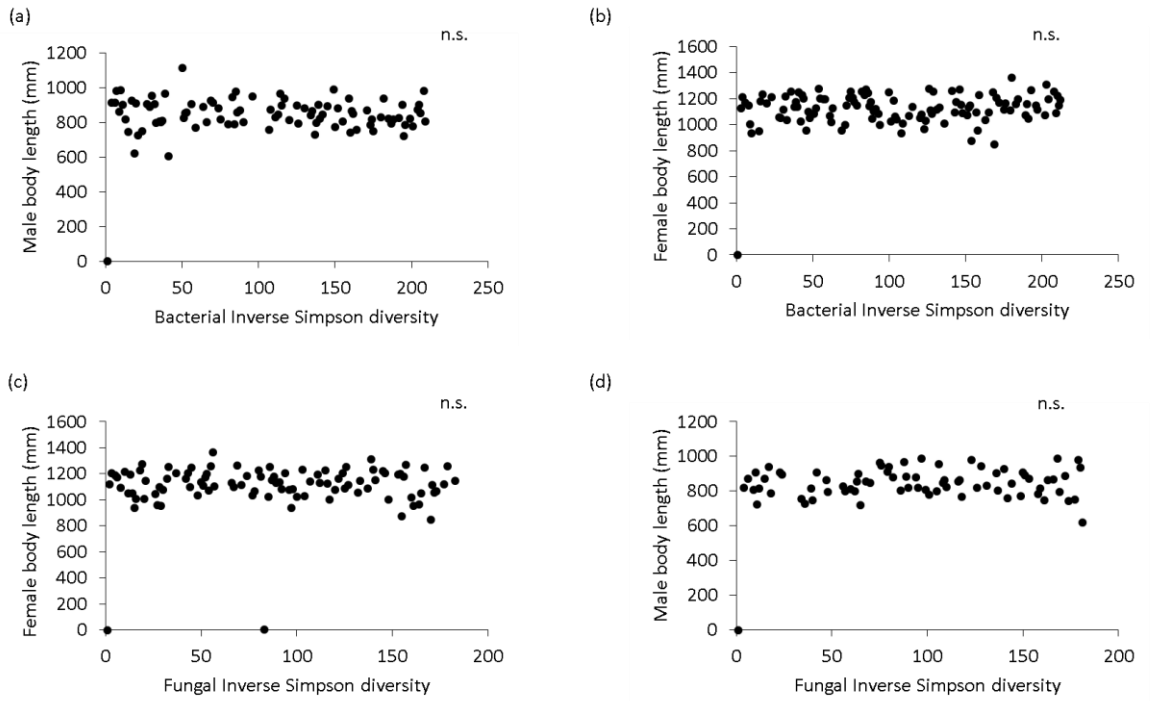

Fig.S5.3 OTUs which were highly related with chrysanthemum shoot biomass and with an average relative abundance over 1%. R and *P*-values following Pearson correlations are presented on each figure.

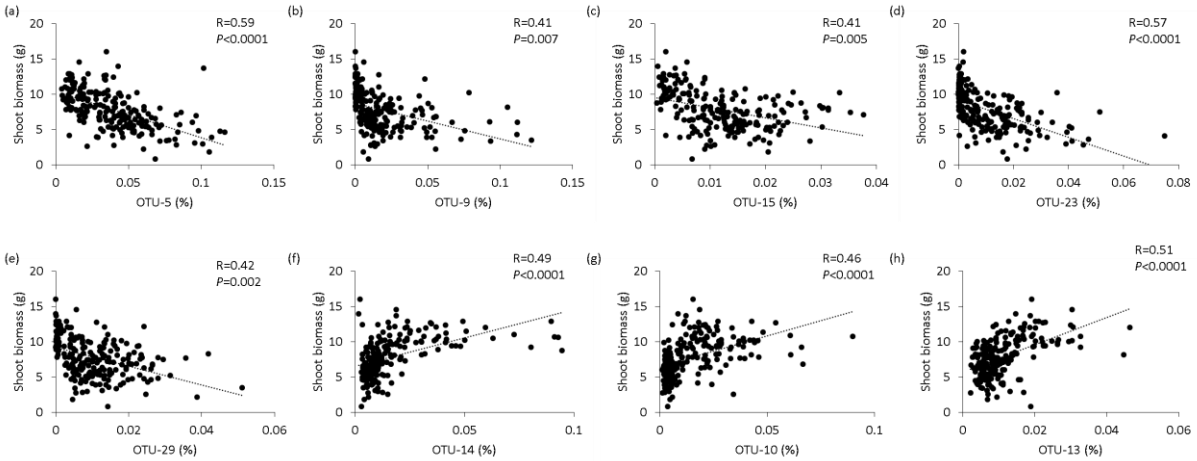

Table S5.3 Chrysanthemum growth-correlated OTUs. R following a Pearson correlation is presented for each OTU, the positive and negative of R indicate the positive and negative correlation between OTU and chrysanthemum biomass, respectively. "Uni" in the genus name indicates unidentified.

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