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Steering root microbiomes of a commercial horticultural crop with plant-soil feedbacks

Hai-kun Ma[a,b], Ana Pineda[a], S. Emilia Hannula[a], Anna M. Kielak[a], Syahida Nindya Setyarini[a], T. Martijn Bezemer[a,b,*]

[a] Netherlands Institute of Ecology (NIOO-KNAW), Department of Terrestrial Ecology, P.O. Box 50, 6700 AB, Wageningen, the Netherlands
[b] Institute of Biology, Section Plant Ecology and Phytochemistry, Leiden University, P.O. Box 9505, 2300 RA Leiden, the Netherlands

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ABSTRACT

Plant-soil feedbacks (PSFs) can influence plant performance in natural and agricultural systems but how PSF principles can be applied in agriculture is not well-studied.

In a two-phase PSF experiment, we tested how inoculating soil conditioned by plants into live and sterilized commercial glasshouse soil influences the root-associated microbiome (bacteria and fungi) and biomass of the cut flower chrysanthemum. The conditioned soil inocula were obtained by growing eight grassland species and chrysanthemum individually in soil collected from a commercial chrysanthemum glasshouse, or in soil from a natural grassland.

Inoculation of conditioned grassland soil into sterilized glasshouse soil led to higher plant biomass, to more complex and connected microbial networks and to a lower abundance of the pathogenic fungi *Olpidium* in chrysanthemum roots, than inoculation into live glasshouse soil or inoculation with conditioned live glasshouse soil. Biomass of chrysanthemum was highest in 100% sterilized soil, but in this soil the root-associated microbiome also contained the highest relative abundance of *Olpidium*.

Glasshouse soils are frequently steam-sterilized and our results show that inoculating these soils with desired soil microbiomes can steer the root microbiome in this crop. However, our study also highlights that steering live glasshouse soil with a disease-related microbiome into a healthy state remains challenging.

1. Introduction

Plant-soil feedbacks (PSFs) occur when a preceding plant influences a succeeding plant by altering the biotic and abiotic conditions of the soil in which it was grown (Bever et al., 1997; van der Putten et al., 2013). In agriculture, mono-cropping, the continuous cultivation of the same crop can lead to the build-up of host specialized pathogens in the soil resulting in reduced yields (i.e. negative conspecific PSF, Mazzoleni et al., 2015). Traditional methods, such as crop rotation and cover cropping, reduce the negative effects of mono-cropping as other crop species influence the soil differently and this can result in reduced pathogen loads in the soil (Dias et al., 2015; Wang et al., 2017). A rapidly increasing number of studies is now highlighting the potential of using resources from natural ecosystems (such as plants and soil) to improve the performance of crops (Vukicevich et al., 2016; Pineda et al., 2017; Kleijn et al., 2018; Mariotte et al., 2018). For example, the grass *Lolium perenne* is shown to increase populations of antibiotic producing bacteria, while the grass *Andropogon gerardi* can stimulate the abundance of arbuscular mycorrhizal fungi (AMF) in the soil, which may improve the growth and resistance against soil-borne diseases of the crop that grows later in the soil (Hetrick et al., 1988; Latz et al., 2015). 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., 2018; 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., 2016), 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 PSFs of plant species and soils from natural ecosystems to enhance the productivity of crops or their resistance against diseases.

The root associated microbiome includes two compartments: the rhizosphere (microbes surrounding the roots) and the endosphere (microbes within the roots) (Lundberg et al., 2012; Fitzpatrick et al., 2018). The root associated microbiome contains a much larger number

* Corresponding author at: Institute of Biology, Section Plant Ecology and Phytochemistry, Leiden University, P.O. Box 9505, 2300 RA Leiden, the Netherlands.
E-mail address: T.M.Bezemer@biology.leidenuniv.nl (T.M. Bezemer).

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of microorganisms than the bulk soil (Bakker et al., 2013), and the activities of these microorganisms are essential for plant growth and health, such as provision of protection against pathogens and facilitation of nutrient uptake (Bakker et al., 2013). One important factor that influences the assembly of the root associated microbiome is the order of the species' arrival (Toju et al., 2018). For example, some microbes used in biocontrol are only effective in suppressing soil pathogens when they colonize the host plant before the pathogen (Braun-Kiewnick et al., 2000; Siddiqui et al., 1999). The early colonizer uses the habitat and resources of the plant root and may potentially produce antibiotic compounds, thus creating barriers for the colonization of later arriving pathogens (Wei et al., 2015; Toju et al., 2018). Therefore, it is possible that by inoculating plants with a beneficial microbiome, the chances of pathogen infection will be reduced. Numerous studies have explored the root associated microbiome of both model plants such as *Arabidopsis thaliana* (Lundberg et al., 2012; Schlaeppi et al., 2014), and crop species such as barley (*Hordeum vulgare*) (Bulgarelli et al., 2015), soybean (*Glycine* max) (Rascovan et al., 2016), corn (*Zea mays*) (Aira et al., 2010), wheat (*Triticum aestivum*) (Donn et al., 2015) and rice (*Oryza sativa*) (Edwards et al., 2015). However, to our knowledge, no study has yet described the changes in root-associated microbiomes of a crop in response to inoculation with plant-conditioned soil microbiomes.

Through rhizodeposition plants can actively recruit or suppress particular soil microorganisms and these effects are plant-species specific (Philippot et al., 2013). The microbial pool that is present in the soil in which the plant grows may also influence the composition of the microbial community in the rhizosphere (Berendsen et al., 2012; Bulgarelli et al., 2012). However, several studies have shown that plant species create similar rhizosphere microbiomes in different soils (Miethling et al., 2000; Wieland et al., 2001; Costa et al., 2006). The net impact of the introduced microbiome on the recipient soil will depend, among other things, on the adaptation of the introduced microbiome to the new environment/soil and on the resilience of the recipient microbiome to the introduced microbiome (Mallon et al., 2015; Thomsen and Hart, 2018). 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 (Haas and Défago, 2005; Mendes et al., 2011; Siegel-Hertz et al., 2018). Hence, an important question is whether and to what extent inoculating soil microbiomes into soils with already existing microbiomes will alter the effects of the existing microbiome on the root associated microbiome of the plant, and to what extent this depends on the similarity of the donor and recipient soil. In this study we use two soils, one derived from a natural grassland and another from a commercial glasshouse. We grew a range of different wild plant species that co-occur in the natural grassland in these soils. We then inoculated these plant-conditioned soils into commercial glasshouse soil and tested whether inoculation with these plant-specific soils resulted in different root associated microbiomes of the crop chrysanthemum and how this depends on the soil used to create the inoculum (grassland/glasshouse). We inoculated the plant-specific soils into live and sterilized glasshouse soil and hence could test the influence of the background soil (live/sterilized).

We examined the effects of inoculation on the biomass of chrysanthemum and its root-associated microbiome six weeks after inoculation. Chrysanthemum (*Dendranthema X grandiflora*) is an economically important ornamental in the horticultural industry. Monocropping of chrysanthemum in commercial glasshouses 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 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 soils in which wild plant species had been grown 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; Hanulla et al., 2019). In the current study, the plant-conditioned soil inocula were added to either sterilized glasshouse soil, resembling the situation immediately after steaming, or to live glasshouse soil, which was collected after five cycles (normally the last growth cycles before steaming the soil) of chrysanthemum cultivation. We determined the root associated microbiomes in chrysanthemum plants growing in all combinations of conditioning soil types (natural or glasshouse soil) and background soil types (sterilized or live glasshouse soil). With this design we tested whether wild plant species can be used to cure soils. 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 PSFs in horticulture and may pave the way to new methods that promote crop growth and health (Bakker et al., 2013).

Specifically, we asked four questions, First, will inoculation with soil conditioned by wild plant species enhance chrysanthemum performance compared to inoculation with chrysanthemum-conditioned soil or un-inoculated soil? Second, will the effects of inoculation with plant-conditioned glasshouse soil resemble the effects of inoculation with grassland soil when these soils are conditioned by the same plant species, and how will this depend on whether the background soil is sterilized or not? Third, how will inoculation with soil from different plant species into live glasshouse soil affect chrysanthemum growth? Fourth, which microbial groups in the chrysanthemum root-associated microbiome correlate with chrysanthemum growth?

2. Materials and methods

2.1. Plant material

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

2.2. 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 glasshouse soil (G) collected from commercial chrysanthemum glasshouse.

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 the focal plant, chrysanthemum (CH). In the test phase, the conditioned soil was used as inoculum (10%) and mixed with either 90% sterilized glasshouse soil (S) or 90% live glasshouse soil (L). A chrysanthemum cutting was then planted in each pot, and shoot biomass and the root-associated microbiome were determined. The experimental design is shown in Fig. 1.

2.2.1. Phase I: conditioning phase

For the conditioning phase, field soil was collected (5–20 cm deep) in April 2017 from a 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. Glasshouse sandy soil was collected in April 2017 from a commercial chrysanthemum glasshouse (Brakel, The Netherlands), the soil already had five cycles of chrysanthemum cultivation when collected. Pots

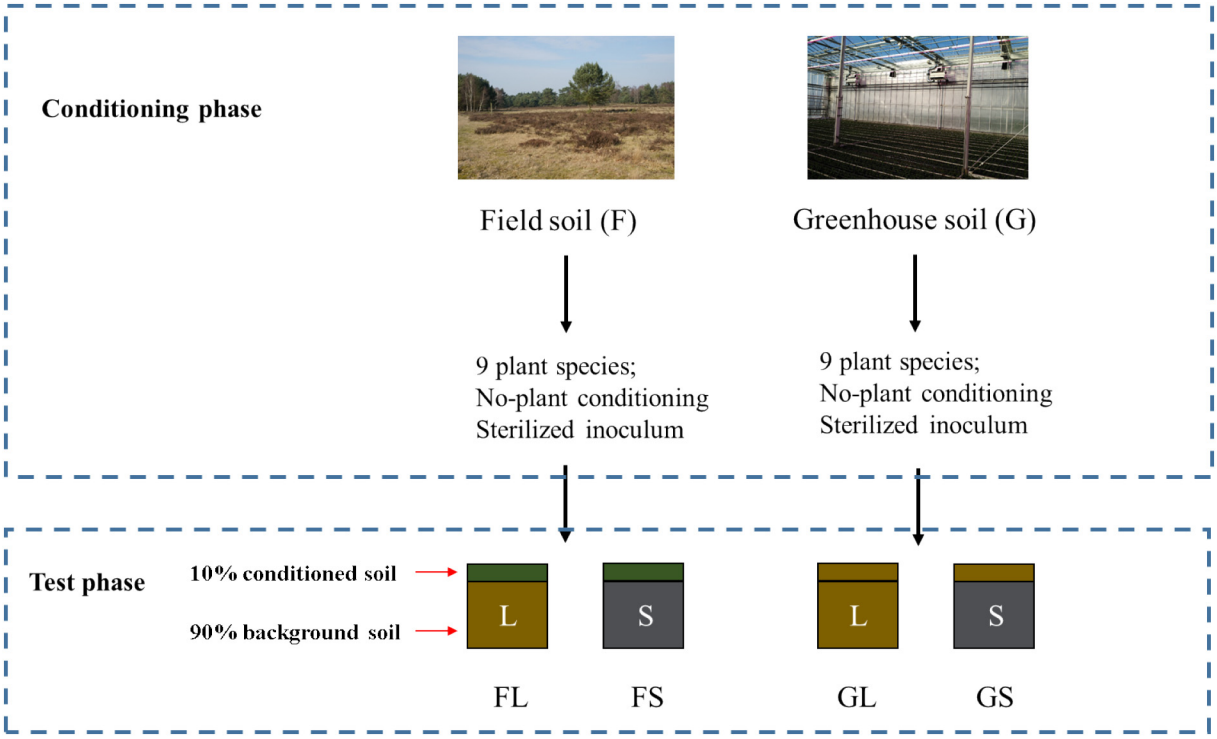

Fig. 1. Overview of the experimental design. The photos show the grassland and the commercial glasshouse where soil was collected. "F" represents field soil, "G" represents glasshouse soil, "L" represents live glasshouse soil, "S" represents sterilized glasshouse soil.

(13 × 13 × 13 cm) were filled with 1.6 kg of either field soil or glasshouse 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 (16 h/8 h, light/dark). In each pot, filled with either field soil or glasshouse soil, five one-week-old seedlings were 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 film. We also included a set of pots with field soil or glasshouse soil that were not planted but kept in the same glasshouse (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 glasshouse 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. Glasshouse soil did not negatively affect the performance of the conditioning plant species, the plants grown in field soil and in glasshouse soil appeared similar. Ten weeks after transplantation, the plants were removed from each pot, fine 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. A subset of the no-plant control soil and the glasshouse (bulk) soil was sterilized using gamma irradiation (> 25 K Gray, Isotron, Ede, The Netherlands). This soil was used as sterilized no-plant conditioning inocula in the test phase.

2.2.2. Phase II: test phase

In the test phase, 1 L pots (11 × 11 × 12 cm; length × width × height) were filled with a homogenized mixture of 10% soil inoculum (plant-conditioned field soil or plant-conditioned glasshouse soil) and 90% background soil. The background soil was live glasshouse soil (L) or sterilized glasshouse soil (S). In total, there were 440 pots: [(8 wild plant species + chrysanthemum + no-plant control + sterilized no-plant conditioning) × 2 conditioning soil types × 2 background soil types × 10 replicates]. Two chrysanthemum cuttings (without roots) were planted in each pot as preliminary work showed that not all cuttings establish properly with this method. Prior to planting, the soil in each pot was watered and 100 ml half-strength Hoagland nutrient solution was added (Hoagland and Arnon, 1939). 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 from 1.2 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 glasshouse with the same conditions as described for the conditioning phase.

Six weeks after the rooting phase, chrysanthemum 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 lightly washed over a sieve (2 mm mesh) using tap water until there was no visible soil attached to the roots. The roots were then freeze-dried and stored at −20 °C to be used for root-associated microbiome analysis. For plants grown in live or sterilized background soil inoculated with conditioned field soil inocula, a representative sample of each root (fine roots and thicker roots) was kept in 70% ethanol for evaluation of the colonization of the roots by arbuscular mycorrhizal fungi (AMF).

2.3. Root colonization by AMF

The roots were first cleared in 10% KOH at 95° for 10 min. Then, roots were stained in a mixture of 20 ml ink (5% Scheaffer black ink) and 380 ml vinegar for 8 min at 80–90 °C. The stained roots were mounted on slides and the colonization of AMF was visually evaluated using the microscopic intersection methods described in McGonigle et al. (1990). We scored 100 root intersections per root sample for the presence and absence of hyphae, vesicules, arbuscules and the structure of non-AM fungi.

2.4. Microbial DNA extraction

In total, root microbiomes of 220 samples were analyzed (5 replicates per treatment). Before extracting DNA, all freeze-dried roots were ground using TissueLyser II, QIAGEN. DNA was extracted from 40 mg powdery freeze-dried root material 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 and 400 ng/ul of DNA. We then carried out PCRs using the 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-blockers were used to block plant DNA (Lundberg et al., 2013). For the PCRs 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, and fungi of 219 samples (see Supplementary Information). Both fungi and bacteria were sequenced in two separate MiSeq PE250 runs each. The mock community was used in each run to verify the comparability of the data. The samples were sequenced at McGill University and the 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 analyzed 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 (Bengtsson-Palme et al., 2013). All sequences created in this study are deposited in ENA with accession number PRJEB35234.

2.5. Standardization of sequencing data

For bacterial data, the total number of reads per sample ranged from 1467 to 85,096, samples with total number of reads < 8000 were removed. OTUs with a total number of reads < 3 in the dataset were also removed. For each sample, the abundance of each OTU was then transformed by dividing it by the total amount of reads per sample (McMurdi and Holmes, 2014). Further, OTUs with a relative abundance of < 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. S1a,b.

The total number of fungal reads per sample ranged from 1 to 9701 because very few fungi were present in the root associated microbiome resulting in strong co-amplification of root material from chrysanthemum roots. Samples with < 140 reads were removed from the dataset. OTUs with < 3 reads in the dataset were then removed. For each sample, abundance of each OTU was transformed by dividing it by the total amount of reads per sample (McMurdi and Holmes, 2014). OTUs with abundance < 0.0069 were then removed. The relationship between the total number of fungal reads and the total number of OTUs before and after the standardization is shown in Fig. S1c,d. The transformed abundance data were used for all analyses of the root microbiome. Because the limited number of fungal reads that was recovered, the fungal data set was not further analyzed. However, the vast majority of the fungal reads were annotated as the pathogen *Olpidium*, and this corresponded with the microscopic observations. Hence the relative abundance of *Olpidium* is presented in the Results section.

2.6. Statistical analysis

Before conducting analyses, data were checked for homogeneity of variance and normality was confirmed by inspection of the residuals. Arcsine square root transformation was performed for the relative abundance of bacterial groups that were not normally distributed (Sokal and Rohlf, 1995). The effects of conditioning (all inocula treatments, including sterilized inocula, no-plant inocula), field or glasshouse soil, and live or sterilized background soil, on plant shoot biomass were examined using a linear mixed model. In the model, inoculum type, field or glasshouse soil and live or sterilized soil were defined as fixed factors, and soil replicate as random factor. Tukey *post-hoc* tests were used for pairwise comparisons between soil combinations. For each soil 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, live no-plant inocula and chrysanthemum conditioned inocula. Post hoc Dunnet tests were used to compare each inoculum effect with the controls. The same statistical analyses were also performed to test the effects of inoculum type, field or glasshouse soil and live or sterilized soil on the relative abundance of bacterial phyla, bacterial diversity and the relative abundance of *Olpidium*, the percentage of root colonization by AMF, and the percentage of root colonization by non-AM fungi.

Simpson diversity was calculated and Pearson correlations were used to determine the relationship between bacterial diversity and plant shoot biomass in the four soil combinations.

Analysis of sequencing data: Permutational multivariate analysis of variance (PERMANOVA) was used to test whether bacterial communities were significantly influenced by inoculum type, conditioning soil type and background soil type. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity 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 dissimilarity between each treatment.

Network analysis: Co-occurrence network analysis was performed to visualize the interactions among bacterial OTUs (Barberán et al., 2012). Spearman Rank correlations were used to determine non-random co-occurrences. For this, only dominant OTUs which occurred in > 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.

To explore whether the relative abundance of particular bacterial OTUs was related to shoot biomass, Pearson correlations were used.

Table 1
Effects of plant and soil treatments on chrysanthemum shoot biomass, and root-associated bacterial diversity.

Factor	Shoot biomass		Bacterial diversity	
	df	F-value	df	F-value
Conditioning (G/F)	1, 180	52.85***	1, 80	1.53
Background (L/S)	1, 216	554.92***	1, 87	29.65***
G/F × L/S	1, 216	93.27***	1, 87	0.13
GL - FL		1.29		
GL - GS		−9.83***		
GL - FS		−20.75***		
FL - GS		−10.51***		
FL - FS		−23.48***		
GS - FS		−11.53***		
I × G/F	10, 180	1.56	10, 80	1.24
I × L/S	10, 216	7.89***	10, 87	0.72
I × G/F × L/S	10, 216	1.48	10, 87	1.21

Inocula:conditioning plant species, no-plant conditioning, and sterilized inocula. G/F: conditioned glasshouse or field soil. L/S: live or sterilized glasshouse background soil. GL: conditioned glasshouse soil inoculated into live glasshouse soil. FL: conditioned field soil inoculated into live glasshouse soil. GS: conditioned glasshouse soil inoculated into sterilized glasshouse soil. FS: conditioned field soil inoculated into sterilized glasshouse soil. Presented are F-values and significance following linear mixed model tests, in the model, inocula, G/F, L/S are fixed factors, soil replicate was included as random factor. df, degrees of freedom. T-values are presented for pairwise comparisons between soil types. Contrasts following a non-significant G/F and L/S interaction were not calculated.

*** $P < 0.001$.

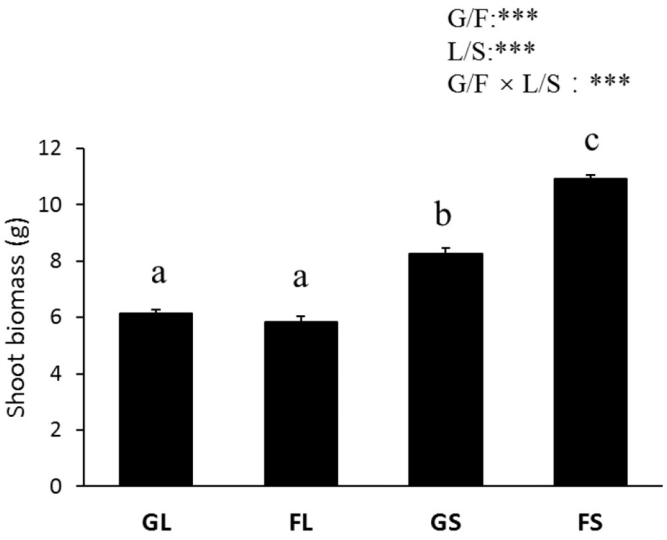

Fig. 2. Mean shoot biomass of chrysanthemum (+1 standard error) in the four soil treatment combinations. "GL" represents conditioned glasshouse soil inoculated into live glasshouse soil. "FL" represents conditioned field soil inoculated into live glasshouse soil. "GS" represents conditioned glasshouse soil inoculated into sterilized glasshouse soil. "FS" represents conditioned field soil inoculated into sterilized glasshouse soil. The overall effects of conditioning soil type (field or glasshouse soil) and the donor soil type (live or sterilized) following a linear mixed model test are shown in the upper part of the figure. Bars with different letters are significantly different at $P < 0.05$.

After FDR (false discovery rate) correction, correlations with the relative abundance of OTUs at $P < 0.05$ were considered significant (Noble, 2009). Consequently, explained variance (R) was higher than 38% for all selected OTUs.

To explore whether the relative abundance of OTUs varied among plant-conditioned soils, differential analyses were performed using DESeq2 package (Love et al., 2014). Taxa with multiple-testing adjusted *P* values smaller than 0.01 were defined as significantly differentially abundant. *P* values were adjusted for multiple testing using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995). All analyses were performed in R (version 3.0.1, R Development Core Team, 2017).

3. Results

3.1. Effects on plant growth

Overall, chrysanthemum shoot biomass was higher in sterilized glasshouse soil than in live glasshouse soil. Chrysanthemum biomass was higher with FS than with GS inocula. There were no significant differences between FL and GL inocula (Table 1, Fig. 2). The plant specific effects of inoculation on chrysanthemum growth depended on the soil combination. For FL, inoculation with soil from *Festuca filiformis* resulted in higher plant shoot biomass than inoculation with chrysanthemum-conditioned soil. The highest shoot biomass of chrysanthemum was observed in the treatments with sterilized inocula (F or G) added to sterilized background soil (S; Fig. S2).

3.2. Effects on the diversity and community structure of the root-associated microbiome

Bacterial diversity positively correlated with plant shoot biomass when the background soil was not sterilized (L; Fig. 3b,c). The composition of the root-associated bacterial community and bacterial diversity were significantly influenced by conditioning plant species, conditioning soil type (F/G) and background soil (L/S glasshouse soil) (Tables 1, 2). Bacterial diversity in chrysanthemum roots was higher in S than L background soil (Table 1, Fig. 3a). There were significant two-way and three-way interactions for the composition of root-associated bacterial communities (Table 2). For FL, inoculation with *Festuca filiformis* and *Rumex acetosella* soil led to higher chrysanthemum root bacterial diversity than inoculation with sterilized soil (Fig. S3).

The NMDS and Ward's cluster analysis revealed a separation between root associated bacterial communities from field and glasshouse 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. 4a,b). The community structure of bacterial communities was not influenced differently in a consistent way by conditioning plant species in the different treatments (Fig. 4b, Fig. S5). Network analysis showed that microbiomes in the FS treatment were more complex than the ones from the other three soil combinations. Microbiomes in the FS treatment were characterized by a higher number of nodes, edges, and connections per node (average degree) (Fig. 4c, Table S1).

3.3. Effects on the composition of root-associated bacterial communities

In the root-associated microbiome of chrysanthemum, Proteobacteria, Actinobacteria, Patescibacteria, Bacteroidetes and Cyanobacteria were the most abundant bacterial phyla (Fig. S4a). Inoculation with glasshouse (G) soils led to a higher relative abundance of Proteobacteria than inoculation with field (F) soils (Table S2, Fig.S4a). In sterilized background soil (S), the relative abundance of Patescibacteria was lower, and the relative abundance of Actinobacteria, Chloroflexi, Verrucomicrobia, and Armatimonadetes was higher compared to live background soil (L). Except for Actinobacteria, these patterns were stronger in the FS treatment (Table S2, Fig.S4). The relative abundances of Bacteroidetes, Acidobacteria and Firmicutes changed but only in FS, 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.

The differences in bacterial phylum abundances between different

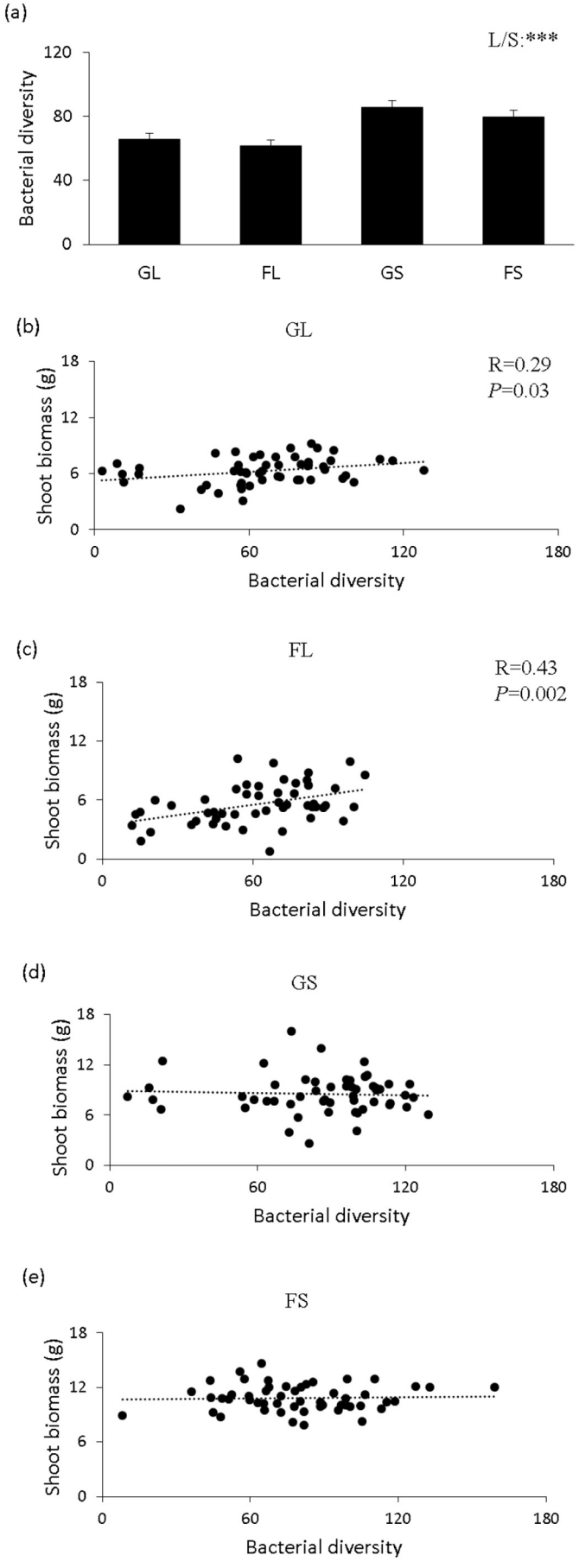

Fig. 3. Bacterial Simpson diversity in the root-associated microbiome of chrysanthemum in the four soil treatments (a) and their relationship with plant shoot biomass in four soil treatments (b-e). In panel a, means are presented, and error bars depict 1 standard error. Statistics of the overall effects following a linear mixed model are presented in the upper part of the figure, only significant effects are shown. "GL" represents conditioned glasshouse soil inoculated into live glasshouse soil. "FL" represents conditioned field soil inoculated into live glasshouse soil. "GS" represents conditioned glasshouse soil inoculated into sterilized glasshouse soil. "FS" represents conditioned field soil inoculated into sterilized glasshouse soil.

Table 2
Effects of plant and soil treatments on the composition of bacterial OTUs.

Factor	Bacteria		
	df	F-value	R^2
G/F	1, 163	36.19***	0.10
L/S	1, 163	74.85***	0.20
I × G/F	10, 163	2.18***	0.06
I × L/S	10, 163	1.66***	0.04
G/F × L/S	1, 163	20.09***	0.05
I × G/F × L/S	10, 163	1.50**	0.04

Data were analyzed using PERMANOVA. Inocula: conditioning plant species, no plant conditioning and sterilized inocula. G/F: conditioned glasshouse or field soil. L/S: live or sterilized glasshouse soil. df: degree of freedom, F-values, significance and explained R^2 are presented.

** Significance: $P < 0.01$.

*** Significance: $P < 0.001$.

plant-conditioned inocula were mainly due to the distinct phylum composition in 100% sterilized soil. Addition of sterilized inocula to sterilized background soil led to a lower relative abundance of Actinobacteria and Acidobacteria, and a higher relative abundance of Cyanobacteria, Chloroflexi, and Armatimonadetes compared to addition of plant-conditioned inocula (Fig. S4a,b).

The fungal community in chrysanthemum roots consisted mainly of one phylotype of Olpidiomycota, namely the *Olpdium brassicae/virulans* complex. The relative abundance of *Olpidium* sp. in the root associated microbiome was lower in the FS treatment than in the other three treatments. Chrysanthemum roots contained a higher relative abundance of *Olpidium* in the GS than in the GL treatment (Table S2, Fig. 5a).

Roots of chrysanthemum in the GS treatment with inocula from *Lolium perenne*, *Anthoxanthum odoratum* and *Achillea millefolium* had lower relative abundances of *Olpidium* sp. (Fig. 5a). For *Lolium perenne* inocula, the same effect was also significant when compared to chrysanthemum conditioned inocula (Fig. 5a). There was a weak significant negative relationship between the relative abundance of *Olpidium* and chrysanthemum shoot biomass but only in background field soil (Fig. 5c,d) The percentage of non-AM fungi structures, detected with microscopic counting, on plant roots was higher in live than in sterilized background soil (Fig. 5b) and negatively correlated with plant shoot biomass when plants were grown with inocula from field soil (Fig. 5e). The colonization of AMF in plant roots was < 4%. There were no treatment effects on the percentage of AMF colonization, and there was no correlation between the percentage of AMF colonization and plant growth (Fig. S7). More OTUs correlated with plant growth in the FL and GS treatments than in the GL treatment (Table S5). No OTUs significantly correlated with plant growth in the FS treatment (Table S5).

4. Discussion

The plant microbiome is composed of active microorganisms that confer plant resistance against biotic (Berg et al., 2014) and abiotic stresses (Yuan et al., 2016). Establishing a healthy microbiome or shifting a disease-related microbiome to a healthy state through PSF

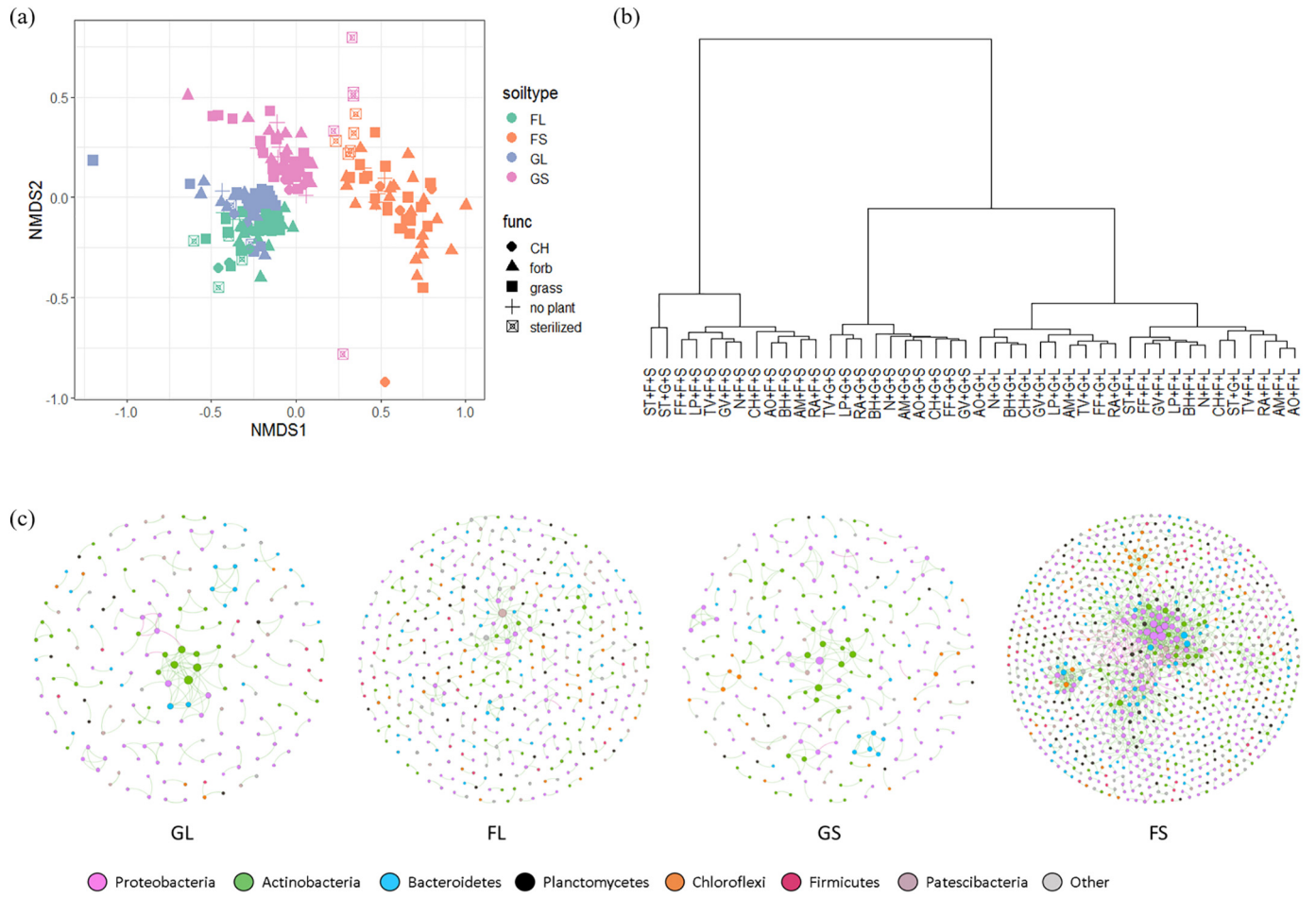

Fig. 4. Non-metric multidimensional scaling (NMDS) plot performed on taxonomic profile (OTU level for 16 s DNA) of root-associated bacteria (a); hierarchical cluster analysis of Bray-Curtis similarities between each treatment on root-associated bacteria (b), and the network co-occurrence analysis of chrysanthemum root-associated microbial communities in the four soil combinations (c). For NMDS plots, four types of conditioning soil and background soil combinations are presented in different colors. The functional groups of conditioning plant species are highlighted by different shapes. "GL" represents conditioned glasshouse soil inoculated into live glasshouse soil. "FL" represents conditioned field soil inoculated into live glasshouse soil. "GS" represents conditioned glasshouse soil inoculated into sterilized glasshouse soil. "FS" represents conditioned field soil inoculated into sterilized glasshouse soil. In the cluster analysis, the names of the treatments consist of conditioning plant species identity + conditioning soil type + background soil type. The abbreviations for the conditioning plant species are described in the Materials and methods section. "ST" indicates sterilized inocula. "N" indicates no-plant inoculum. "G" indicates glasshouse soil, "F" indicates field soil. "S" indicates sterilized glasshouse soil. "L" indicates live glasshouse soil. In the network co-occurrence analysis a connection stands for a Spearman Rank correlation with a magnitude > 0.7 (both positive and negative) and that is statistically significant ($P < 0.05$ after Bonferroni correction). Red edges indicate negative correlations, green edges indicate positive correlations. Each node represents an OTU, and the size of node is proportional to its number of connections (i.e. degree). Each node is colored at phylum level. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

principles can provide new insights that can be used for sustainable management of agricultural systems (Busby et al., 2017). In this study, we show that glasshouse soil that had five cycles of mono-cropping, had strong negative effects on plant growth compared with sterilized soil, but inoculation with soil from a natural ecosystem did not reduce this negative effect considerably, indicating that rescuing a soil with a disease-associated microbiome is difficult. The inoculated microbiome established much better in sterilized soil, FS was the best soil combination for chrysanthemum performance, and also led to the most distinctive microbiome structure. This indicates that soil inoculation will be most successful after steaming the soil.

Plant growth was negatively affected when growing in glasshouse soil, and such negative effects were not reversed by the microbiome inoculation. These results indicate that shifting a disease-associated microbiome in the soil to a healthy one is difficult (Toju et al., 2018). The resilience and resistance of the current community may lead to the returning of the new community to a similar structure even with external disturbance (Lozupone et al., 2012; Griffiths and Philippot, 2013). However, other studies have found that adding 10% disease suppressive microbiome to 90% of a disease-related microbiome was enough to successfully transfer disease suppressive properties to this microbiome (Haas and Défago, 2005; Mendes et al., 2011; Siegel-Hertz et al., 2018). It is important to note that in those studies, the 10% disease suppressive soil specifically suppressed a target pathogen. This specific disease suppression is likely due to the activity of a few microbes that are antagonistic to the target pathogen, and this is transferrable by adding a small amount to conducive soil (Schlatter et al., 2017). In our study, the conditioned inocula did not consist of soil with specific disease suppression to a target pathogen in the glasshouse soil, although previously we showed that inoculation of these conditioned soils into sterilized soil can lead to increased biomass when plants were also exposed to the soil pathogen *Pythium* (Ma et al., 2017, 2018). An interesting future direction could be to first steer the soils into a disease supressiveness state against the target pathogens in glasshouse soil prior to inoculation. This can be achieved by adding these diseases to the soil during the conditioning phase, and increasing the number of growth cycles during the conditioning phase. Interestingly, several plant species from natural ecosystems such as the grasses *Lolium perenne*

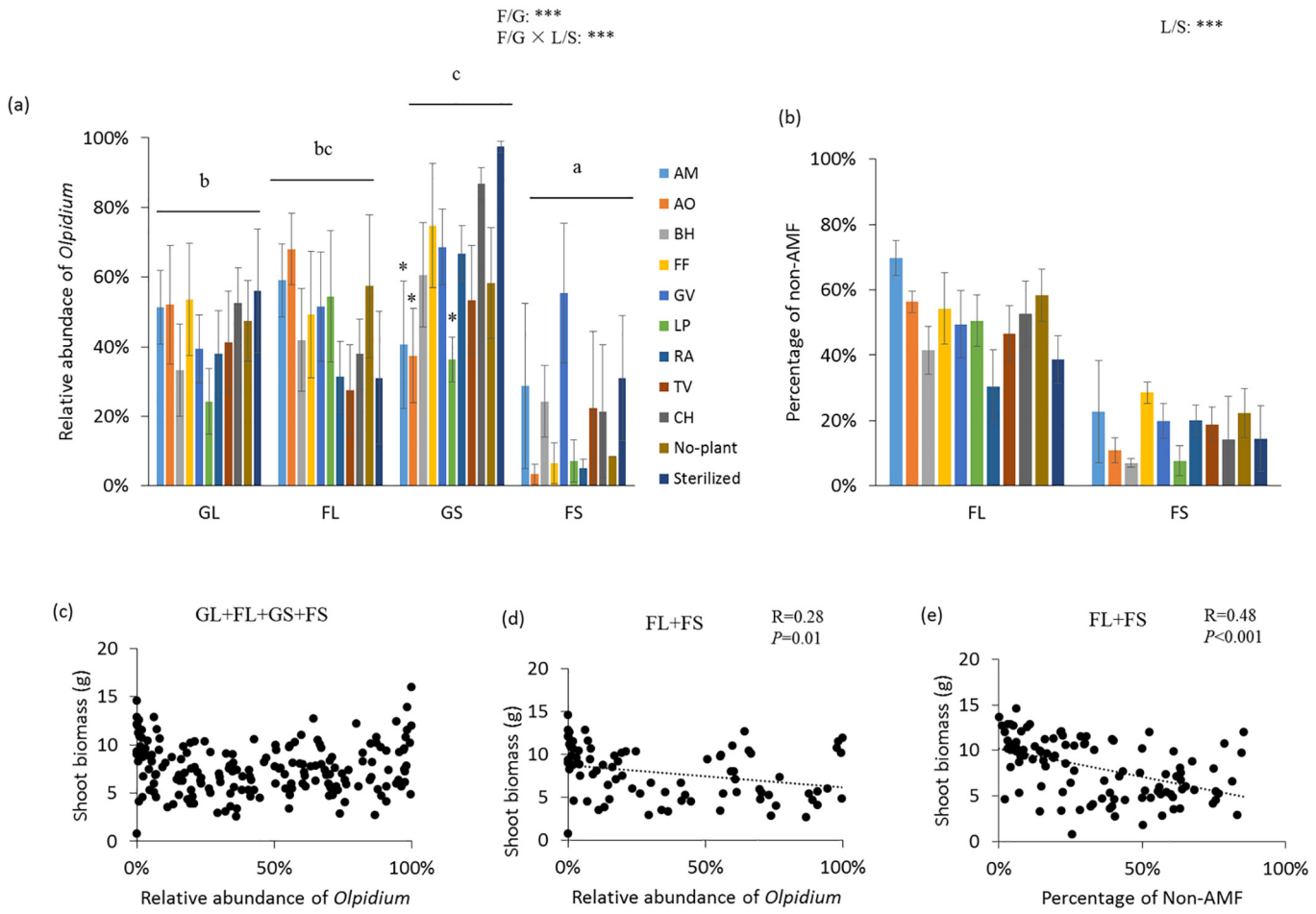

Fig. 5. The relative abundance of *Olpidium* in the different soil treatments based on Illumina sequencing (a); the percentage of non-AMF in different soil treatments based on morphology (b); the relationship between the relative abundance of *Olpidium* and plant shoot biomass (c); the relationship between relative abundance of *Olpidium* and plant shoot biomass in FL and FS (conditioned field soil inoculated into live and sterilized glasshouse soil) (d); and the relationship between the percentage of non-AMF with plant shoot biomass in FL and FS (conditioned field soil inoculated into live and sterilized glasshouse soil) (e). In panel a, b, values are means, and error bars depict ± 1 standard error. The statistics of the overall effects are based on the arcsine square-root transformed data, and presented in the upper part of the figure, only significant effects are shown. Different letters above each group of bars indicate that these groups significantly differ. "*" indicates significant difference when compared to sterilized inocula ($P < 0.05$). "+" indicates significant difference when compared to chrysanthemum-conditioned inocula. "GL" represents conditioned glasshouse soil inoculated into live glasshouse soil. "FL" represents conditioned field soil inoculated into live glasshouse soil. "GS" represents conditioned glasshouse soil inoculated into sterilized glasshouse soil. "FS" represents conditioned field soil inoculated into sterilized glasshouse soil. The conditioning plant species are represented by their abbreviations; the full names are described in the Materials and methods section. "No-plant" indicates no-plant inoculum. "Sterilized" indicates sterilized inoculum.

and *Anthoxanthum odoratum* are less susceptible to soil pathogens than many forbs and legumes (Mills and Bever, 1998; Stiles et al., 2007; Bithell et al., 2011). These plant species make good candidates to condition the soil as they may increase the population of beneficial soil microbes in the soil in the presence of pathogens (van Dam, 2009; Wei et al., 2015).

Inoculating conditioned soil into sterilized soil led to the most distinct root microbiome composition. This is not surprising as an empty soil environment, without competition, may support colonization of the roots (Ndoye et al., 2013; Leifheit et al., 2014; Toju et al., 2018). However, adding a microbial community into sterilized soil may also lead to the proliferation of pathogens. Indeed, plants had a higher relative abundance of *Olpidium* in the GS than in the GL treatment. Soil sterilization may have killed also the beneficial microbes allowing the pathogens to proliferate (Thuerig et al., 2009; Schlatter et al., 2017; Bonanomi et al., 2018). It is important to notice that our sequence data is expressed as relative abundances and hence this does not provide information about disease severity. However, the absolute data obtained from the microscopic observations on the percentage of non-AMF structures on plant roots resembled the relative abundance pattern for *Olpidium* in the glasshouse soil. Moreover, the relative abundance of *Olpidium* and the percentage of non-AM fungi were negatively correlated with plant biomass. Hence, this suggests that the severity of the pathogen *Olpidium* was higher after inoculation into sterilized background soil. The dominant pathogenic fungi *Olpidium* is a broad range pathogen. It can transfer viruses and infection of the pathogen can lead to reduction in roots and discoloration of roots (Singh and Pavgi, 1977; Lay et al., 2018). Our study shows that mono-cropping of chrysanthemum can lead to accumulation of *Olpidium* in the roots. On the other hand, inoculation also led to increases in the relative abundance of bacterial phyla such as *Chloroflexi, Verrucomicrobia*, *Armatimonadetes*, that were positively correlated to plant growth. A large proportion of the bacteria in the phylum of *Chloroflexi* are known to acquire energy and fix CO_2 through photosynthesis (Klappenbach and Pierson, 2004; Hanada et al., 1995). A previous study found that *Chloroflexi* was more abundance in an intercropping than in a mono-cropping system (Li and Wu, 2018). *Verrucomicrobia* has also been found in the rhizosphere of other crops, such as leek (Da Rocha et al., 2013) and maize (Aguirre-von-Wobeser et al., 2018), and was found to form beneficial interactions with maize (Aguirre-von-Wobeser et al., 2018). Interestingly, in

previous studies *Chloroflexi* and *Verrucomicrobia* were reported as being enriched in soils that were suppressive against fungal pathogens (Sanguin et al., 2009; Xiong et al., 2017).

The most connected and complex microbial community was found in the FS treatment, however, the highest plant growth was observed when plants grew in 100% sterilized soil. This suggests that pathogens may have been present in the soils that we used during the conditioning phase. Our results are in sharp contrast with a previous study (Badri et al., 2013). In our system, plants received high levels of fertilization, a situation that may negatively influence the symbiotic relationships between plants and soil microbes (Morgan et al., 2005). For example, high concentrations of nitrogen or phosphorus in the soil can directly reduce the growth and activity of AMF (Oehl et al., 2004), and can inhibit the formation of symbiosis between AMF and the host plant (Kiernan et al., 1983; Nouri et al., 2014). Several studies have reported that chrysanthemum is colonized by AMF (Sohn et al., 2003; D'Amelio et al., 2011; del Mar Montiel-Rozas et al., 2016). However, the colonization of AMF in our study was < 5%, indicating that this symbiosis was inhibited. The benefits provided by other microbes to plants may also be lost under high nutrient supply (De Deyn et al., 2004). This has been demonstrated for example for plant growth promoting bacteria, such as *Pseudomonas* spp. (Carlier et al., 2008; Zabihi et al., 2011). However, some bacterial strains can increase plant growth by facilitating the nutrient uptake of plants even at high nutrient supply (Shaharoona et al., 2008; Adesemoye and Kloepper, 2009; Miransari, 2011). Further studies are needed to compare the effects of soil inoculation on chrysanthemum at different levels of fertilization to infer whether the current fertilization practice used in commercial chrysanthemum glasshouses overrules the potential growth promoting effects of inoculated soil communities on chrysanthemum.

5. Conclusions

In conclusion, this study examining the potential use of PSFs and inoculation of microbiomes in glasshouse soils highlights that steering a disease-related microbiome into a healthy one is challenging. The most distinct microbiome networks in chrysanthemum roots were found in the FS treatment. Plant biomass was highest in un-inoculated sterilized soil, but in this soil, pathogens were also most abundant, potentially leading to pathogen outbreaks later on, and hence sterilization without inoculation may not be a sustainable strategy. Future studies should further unravel how to establish healthy microbiomes on crop plants and the longer-term consequences of these microbiomes for crop health.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary data

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