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RESEARCH

Suppression of *Phytophthora* on *Chamaecyparis* in Sustainable Horticultural Substrates Depends on Fertilization and Is Linked to the Rhizobiome

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ABSTRACT

Nature management residues (i.e., biomass generated from the management of nature reserves) are promising peat alternatives for horticultural substrates and may have a positive effect on disease suppression because of their microbiological characteristics. Moreover, addition of fertilizer may also affect the rhizosphere microbiome and, accordingly, disease suppression. In this study, we determined the effect of two management residues in horticultural substrates (i.e., chopped heath and acidified soft rush) and two fertilization regimes (i.e., pure nitrogen fertilizer and compound fertilizer) on the suppression of *Phytophthora* spp. on *Chamaecyparis lawsoniana*. The bacterial and fungal rhizosphere community was characterized using 16S ribosomal RNA and internal transcribed spacer 2 gene metabarcoding. Soft rush with a compound fertilizer (R2) and chopped heath with a pure nitrogen fertilizer (H1) showed a disease-suppressive effect and showed the largest shifts in microbial community composition compared with peat-based substrates. The disease-suppressive treatments showed differences in their microbial communities. Different genera associated with described biocontrol agents for *Phytophthora* spp. were found in higher amounts in those treatments. *Aspergillus* and *Trichoderma* spp. were highly abundant in H1, while *Actinomadura* and *Bacillus* spp. had a high abundance in R2. In addition, the relative abundances of 24 bacterial and 9 fungal genera were negatively correlated with disease severity. Several of those genera, including *Bacillus*, *Chaetomium*, and *Actinomadura*, were significantly more abundant in one of the disease-suppressive treatments. This study shows that disease suppressiveness in sustainable horticultural substrates is dependent on fertilization and can be linked to changes in the microbial rhizosphere communities.

Keywords: disease suppressiveness, fertilization, microbiology, nature management residues, sustainable horticultural substrates

Peat is a preferred horticultural substrate because of its favorable physical and (bio)chemical characteristics (Michel 2010; Schmilewski 2008). However, environmental concerns regarding damage to valuable habitats and high carbon emissions during peat extractions have highlighted the negative ecological footprint of this material (Bonn et al. 2016). For that reason, sustainable alternatives for peat in horticulture are needed.

The use of residual biomass may be a promising avenue in the search for sustainable peat alternatives. Various types of residual biomass, including composts, coir fiber, wood fiber, and pine bark, have been studied for their use in horticultural substrates (Barrett et al. 2016; Gavilanes-Terán et al. 2017; Gruda 2012; Kleiber et al. 2012; Zhang et al. 2013; Zhong et al. 2018). For calcifuge ornamental plants, nature management residues (i.e., biomass generated from the management of nature reserves) have suitable

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physicochemical and (bio)chemical properties to be used as peat alternatives (Miserez et al. 2020; Vandecasteele et al. 2021; Wissner et al. 2017). Miserez et al. (2020) showed that management residues such as sods and chopped biomass from heathland management can replace 40% of peat in substrates for calcifuge ornamental plants without loss of plant quality.

An additional benefit of nature management residues as sustainable peat alternatives may be their microbiological characteristics. The horticultural substrate is one of the main drives for the rhizosphere microbiome and the interaction between plants and their rhizosphere microbiome is critical to plant growth and health (Baudoin et al. 2003; Chaparro et al. 2012; Quiza et al. 2015). Vandecasteele et al. (2021) showed that management residues have a higher microbial biomass than peat-based substrates. In addition, management residues have a higher fungal/bacterial ratio. A high microbial biomass and fungal/bacterial ratio may be linked to higher disease suppression (Bongiorno et al. 2019; De Corato 2020; Neher et al. 2022). Additionally, management residues may support beneficial microorganisms for the plant (Miserez 2021; Pot et al. 2022). Beneficial microorganisms such as plant-growth-promoting rhizobacteria and fungi and biocontrol agents may enhance plant growth and resistance to plant pathogens when present in the rhizosphere (Berendsen et al. 2012). However, the microbiological mechanisms involved in plant growth and health promotion are likely to be plant and pathogen specific (Berendsen et al. 2012; Bonanomi et al. 2010; Pascale et al. 2020).

In addition to the substrate, fertilization also has an important effect on plant growth and health. The use of inorganic fertilizers is a common practice to optimize plant growth (Ali et al. 2021). In addition, fertilization also affects suppression of plant pathogens and disease severity, although the effects are not straightforward to predict. Nitrogen (N) availability as well as the form of N (ammonium or nitrate) may affect the susceptibility of plants to pathogens, yet the effects are dependent on the pathogen species (Veresoglou et al. 2013). Potassium (K) fertilization may decrease disease severity, while the effect of phosphorus (P) fertilization on disease severity is indecisive (Amtmann et al. 2008; Dordas 2008). The balance between nutrients may be another factor complicating the outcome; for example, Akgül and Erkiliç (2016) showed that pure N fertilizers and compound fertilizers (NPK) both have a different effect on disease suppression. The effects of different fertilizers on plant growth and health may be linked to chemical changes in the substrate. However, fertilizers also affect the rhizosphere microbiome. Both the microbial community composition and the microbiome functioning in the rhizosphere may be affected by N, P, and K fertilization (Beltran-Garcia et al. 2021; Chen et al. 2019; Pan et al. 2014; Revillini et al. 2019; Zhao et al. 2020). Although studies have shown that the effect of fertilizers on plant growth and quality is substrate dependent (El-Naggar and El-Nasharty 2009; Mohamed 2018; Shalizi et al. 2019; Youssef 2014), it is not clear how the interaction between fertilization and substrate affects the rhizosphere microbiome and disease suppressiveness.

Chamaecyparis lawsoniana 'Ellwoodii' (Lawson's cypress) is one of the most important conifers in ornamental horticulture and is widely grown in Europe and North America (Robin et al. 2015). *Chamaecyparis* spp. commonly suffer from infections by root-borne pathogens of the *Phytophthora* genus (Werres et al. 1997). *Phytophthora* spp. cause root rot in a broad range of host plants, leading to symptoms such as chlorosis, wilting, and plant death (Weiland 2021). Production losses due to these pathogens typically range from 10 to 20% but can mount to 100% (Weiland 2021; Werres et al. 1997). One of the most frequently encountered species of *Phytophthora* in ornamental horticulture is *Phytophthora cinnamomi*, infecting several ornamental genera, including *Chamaecyparis* (Weiland 2021). Biocontrol agents that have been reported to suppress *Phytophthora* spp. include *Trichoderma*, *Bacillus*,, *Fusarium*, *Aspergillus*, *Penicillium*, *Streptomyces*, *Actinomadura*, and *Pseudomonas* spp. (Duvenhage and Kotzé 1993; Macías-Rodríguez et al. 2018; Méndez-Bravo et al. 2018; Stirling et al. 1992; Turnbull et al. 1992; You et al. 1996).

In this study, we determined the effect of the use of management residues in substrates and two fertilization regimes—a pure N fertilizer and a compound fertilizer—on plant growth of *C. lawsoniana* 'Ellwoodii' plants, and on the suppression of a natural infection with *Phytophthora* spp. We hypothesize that management residues will increase plant growth and will promote the suppression of the pathogen, leading to decreased disease severity, depending on the fertilization regime. Moreover, we hypothesize that effects on disease suppression may be attributed to changes in the composition and diversity of the rhizosphere microbiome. Therefore, the bacterial and fungal rhizosphere community was studied using 16S ribosomal RNA (rRNA) and internal transcribed spacer 2 (ITS2) gene metabarcoding.

MATERIALS AND METHODS

Management residues. Two types of management residues were used in this study: chopped soft rush (*Juncus effusus*) and chopped heath. Chopped soft rush straw (abbreviated as R) was collected from a nature conservation area in Huldenberg-Dijlevallei, Belgium and stored outside for 12 months under a plastic cover before sampling. After silage, the material had a pH of 7.86. To decrease the pH, the R was mixed with elemental sulfur in a concentration of 1 g/liter. Chopped heath (abbreviated as H) was received from the military domain of Meeuwen-Gruitrode, Belgium. The chemical properties of these management residues were identified as highly favorable for use in horticultural substrates in high-volume fractions (Vandecasteele et al. 2021) (Supplementary Table S1). The chemical properties were determined based on the European Standards developed by the European Committee for Standardization (CEN). European Standard (EN) numbers refer to the specific standards. Electrical conductivity: pH-H_2O; and Cl, Na, and SO_4 were measured in a 1:5 (vol/vol) water extract according to EN 13038 (CEN 2011b), EN 13037 (CEN 2011a), and EN 13652 (CEN 2001), respectively. NO_3-N + NO_2-N and NH_4-N were measured with a Skalar San++ Continuous Flow Analyzer (Skalar, Breda, The Netherlands). K, Mg, Ca, Fe, Mn, Zn, Cu, and P were extracted (1:5, vol/vol) in 0.5 M ammonium acetate buffered at pH 4.65 (with a 96% acetic acid). The K, Mg, Ca, Fe, Mn, Zn, Cu, and P concentration in the extract was measured by inductively coupled plasma optical emission spectroscopy.

Container trials with *C. lawsoniana* 'Ellwoodii'. A container trial with *C. lawsoniana* 'Ellwoodii' plants was set up which was divided into a growth experiment, in which the growth of the plants was evaluated, and an infection experiment, in which a natural infection with *Phytophthora* was evaluated. An overview of the two experiments can be found in Figure 1.

Three different substrates were used. The two management residues (H and R) were each mixed with peat in a 60:40 vol% ratio. A 100 vol% peat substrate (Agaris, Belgium) was used as a reference (C) (Supplementary Table S1). In addition, two fertilization regimes were applied. In the first fertilization regime (regime 1), pure N was added (KAS at 2.1 g/liter [13.50% NO_3 and 13.50% NH_4] + Agroblen Scotts at 0.22 g/liter [35.00% NH_4]) whereas, in the second fertilization regime (regime 2), the same level of nitrate-N and ammonium-N was added but as controlled release compound fertilizer (5 to 6 M Osmocote exact standard at 3 g/liter [15:9:12 N-P-K + 2MgO + Tris-EDTA {TE}] + 8 to 9 M Osmocote exact

standard at 1.3 g/liter [15:9:11 N-P-K + 2MgO + TE]). Fertilizers were mixed in the three different substrates. The contribution of the management residues to nutrient availability was considered to be nihil (Supplementary Table S1). The combination of three substrates with two fertilization regimes resulted in six treatments: C1, C2, H1, H2, R1, and R2.

The fertilized substrates were put in 0.7-liter pots. Subsequently, a rooted cutting of *C. lawsoniana* 'Ellwoodii' was planted in each pot. For each experiment, 48 biological replicates were planted per treatment, resulting in a total of 288 plants in each experiment.

The plants of both experiments were cultivated on an outdoor container field covered with a groundcover tissue located in Destelbergen, Belgium. During previous experiments on this container field, *Phytophthora* infections occurred naturally. Therefore, we assumed that *Phytophthora* spp. were present at this specific location. *Phytophthora* can persist in the soil for several years and can spread through rainwater. Infection of plants is promoted by wet conditions in the substrate (Davison 1998; Linderman and Benson 2016). The 288 plants of the growth experiment were placed in a double pot to avoid infection with *Phytophthora* spp. (Fig. 1). Plants

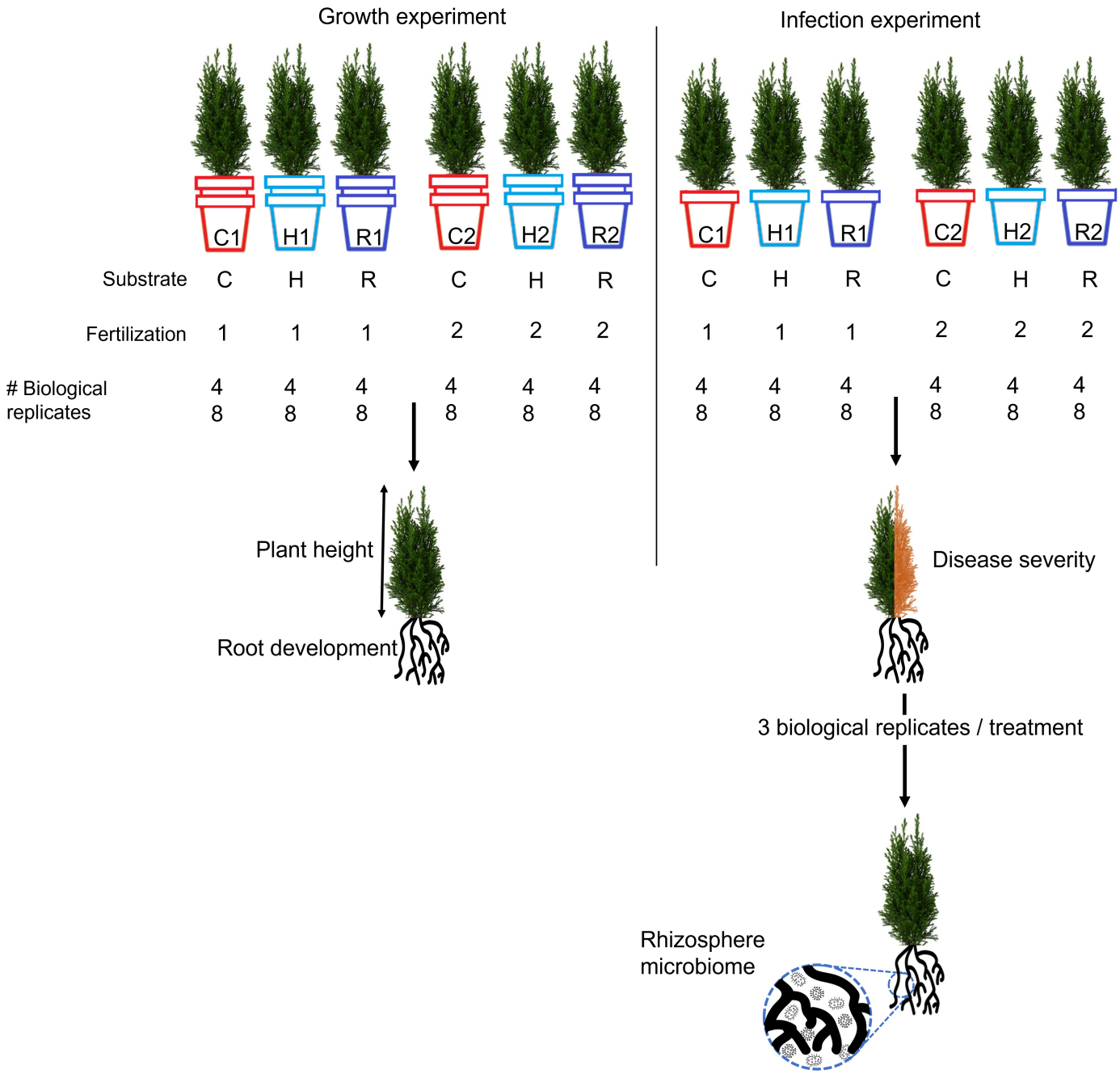

Fig. 1. Overview of the two experiments conducted with *Chamaecyparis lawsoniana* 'Ellwoodii'. In each experiment, three substrates were used: 100 vol% peat substrate (C), 40 vol% peat and 60 vol% chopped heath (H), and 40 vol% peat and 60 vol% soft rush (R). In addition, two fertilization regimes were used: pure nitrogen (regime 1) and controlled-release compound fertilizer (regime 2). For each treatment, 48 biological replicates were planted, resulting in a total of 288 plants for both experiments. All plants were grown on an open-air container field with groundcover tissue. In the growth experiment, plants were grown in a double pot to prevent infection with *Phytophthora* spp. Plant height and root development were measured. In the infection experiment, the disease intensity caused by a natural infection with *Phytophthora* spp. was scored. Three biological replicates per treatment were selected to study the rhizosphere microbiome using 16S ribosomal RNA and internal transcribed spacer 2 gene metabarcoding.

that are grown in a double pot remain healthy because it prevents infected run-off rainwater from coming into contact with the substrate and with the plant roots. Moreover, it reduces the wet conditions in the substrate, allowing more circulation of air around the roots and better drainage of water. The 288 plants in the infection experiments were cultivated in one pot and, thus, left exposed to run-off rainwater, allowing natural infection with *Phytophthora* spp. At the start of the experiment, none of the plants showed *Phytophthora* infection symptoms such as discoloration or wilting.

Plants were arranged in a completely random design on the container field and followed during the whole growing season (21 weeks). Overhead sprinkler irrigation was used, and the irrigation schedule was based on radiation sum where a radiation sum of 1,250 J/cm² corresponded to 450 liters. No additional fertilizer was applied during the trial.

In the growth experiment, plant height was manually measured with a ruler after 21 weeks. Additionally, root development was evaluated using a scoring system based on the root system that was visible at the surface of the substrate when the pot was removed (Miserez et al. 2019). Depending on the number of visible lateral roots, a score of 1 to 5 was given, where 1 = very few lateral roots, 2 = few lateral roots, 3 = moderate number of lateral roots, 4 = large number of lateral roots all over the substrate surface, and 5 = very large number of lateral roots that cover the whole substrate surface.

In the infection experiment, disease severity was determined using a scoring system based on the area of the plant that was visibly diseased. The plants were given a score of 1 to 4, where 1 = no visible disease, 2 = small area of the plant visibly diseased, 3 = half of the plant visibly diseased, and 4 = whole plant visibly diseased. Symptoms that were taken into account included discoloration, wilting, and plant death (Supplementary Fig. S1) (Böhne 2006). At the end of the experiment, three plants per treatment (18 plants in total) (Supplementary Table S2) were randomly selected for rhizosphere sampling, as described below. In addition, the presence of *Phytophthora* spp. in plant tissue of symptomatic plants was confirmed at the Diagnostic Centre for Plants of Flanders Research Institute for Agriculture, Fisheries and Food by plating onto semiselective PARP medium (Jeffers and Martin 1986), microscopic analysis, and a baiting test for oomycetes based on Vercauteren et al. (2013). One isolate was molecular identified (using ITS sequencing) as *P. cinnamomi* (White et al. 1990).

Sampling and DNA extraction. Rhizosphere samples were taken from 18 selected plants (three biological replicates per treatment) from the infection experiment. The rhizosphere was sampled following Lundberg et al. (2012): loose soil was manually removed from the roots and roots were then placed in a 50-ml tube with 25 ml of phosphate buffer. The tubes were vortexed to release the rhizosphere soil from the roots. To remove plant parts and large sediment, the solution was filtered through a 100-mm nylon mesh cell strainer into a new 50-ml tube. The filtered solution was centrifuged for 15 min at 3,000 × *g* to form a pellet (250 mg) that was considered to be the rhizosphere sample. Samples were stored at −20°C until DNA extraction.

DNA was extracted from each sample using the DNeasy Powersoil Pro Kit (Qiagen, Germantown, MD, U.S.A.), according to the manufacturer's instructions, and stored at −20°C until use for metabarcoding.

16S rRNA and ITS2 gene metabarcoding. Metabarcoding of the bacterial and fungal populations was done on the V3-V4 fragment of the 16S rRNA gene and on the ITS2 gene, respectively, as described in detail by De Tender et al. (2016a). Briefly, the fragments were amplified using an amplification PCR. The bacterial V3-V4 fragment was amplified using the primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 (Klindworth et al. 2013). To amplify the fungal rDNA-ITS2 region, an adapted forward primer of fITS7bis from Ihrmark et al. (2012) (GTGAAT CATCRAATYTTTG) and the ITS4NGSr reverse primer (CAWC GATGAAGAACGYAG) (Tedersoo et al. 2014) were used. Fragments were extended with Illumina-specific adaptors using a dual-index PCR. Mastermixes for all PCRs were prepared using the Kapa HiFi Hotstart ReadyMix (Kapa Biosystems, Wilmington, MA, U.S.A.) according to instructions of the manufacturer. A PCR product clean-up was performed after each PCR step using the CleanPCR reagent kit (MAGBIO, Gaitherburg, MD, U.S.A.). Final libraries were quality controlled by gel electrophoresis. Concentrations were measured using the Quantus double-stranded DNA assay (Promega Corp., Madison, WI, U.S.A.). The final barcoded libraries were diluted to 10 nM and pooled. Resulting libraries were sequenced using Illumina MiSeq v3 technology (2 × 300 bp, paired-end) by Admera (United States) using 30% PhiX DNA as spike-in. Reads are available for download at the NCBI sequence read archive under project number PRJNA809191.

Demultiplexing of the metabarcoding dataset and removal of the barcodes was performed by the sequencing provider. Primers were removed using Trimmomatic version 0.32 (Bolger et al. 2014). Adapters were already removed by the sequencing provider. Quality of the preprocessed sequences was checked using FastQC version 0.11.8 (Andrews 2010). Further processing of the sequences was done using the DADA2 pipeline version 1.16 (Callahan et al. 2015, as described in detail by Joos et al. 2020a). Briefly, low-quality reads were trimmed, sequences were dereplicated, and amplicon sequence variants (ASVs) were inferred based on the parametric model of errors calculated by the algorithm. Inferred sequences were merged and chimeras were removed. This procedure resulted in an average of 57,199 ± 7,846 reads/sample for the bacterial dataset and an average of 38,506 ± 2,560 reads/sample for the fungal dataset (Supplementary Fig. S2). Taxonomy was assigned by the SILVA database v132 (bacteria) (Glöckner et al. 2017; Quast et al. 2012; Yilmaz et al. 2013) and UNITE database v020219 (fungi) (Nilsson et al. 2018). A bacterial and fungal sequence table was constructed.

Downstream analysis and statistics. For the metabarcoding data, all analyses were done for both the bacterial and fungal sequence tables. Low abundant reads were removed by removing ASVs with less than three counts per million in at least three samples from the sequence tables. Filtered sequence tables were then used for further analysis. First, the nonrarefied sequence table was used to calculate the Shannon diversity index using the diversity function of the vegan package (version 2.5.7) in R (version 4.0.4) (Oksanen et al. 2020). To find significant differences in mean bacterial and fungal diversity between the treatments, a linear model was used, with main effects for substrate and fertilization and the infection–fertilization interaction after checking the assumptions. P values < 0.05 were considered significant. Second, absolute ASV counts were transformed to relative abundances. From this ASV table, a dissimilarity matrix (based on the Bray-Curtis dissimilarity index) was calculated. Homogeneity of the variances was checked on this dissimilarity matrix using the betadisper function. The effect of substrate and fertilization was studied by doing a permutational multivariate analysis of variance (PERMANOVA) analysis on the dissimilarity matrix. To visualize the observed differences, principal coordinate analysis (PCoA) on the dissimilarity matrix was done. Third, the composition of the top 10 most abundant genera was studied based on the ASV table with relative abundances. Indicator species analysis was performed on the ASV table with relative abundances at genus level using the indicspecies package (version 1.7.9) (De Cáceres and Legendre 2009). Fourth, the

effect of treatment on abundance was tested using the edgeR package (version 3.32.1) (Robinson et al. 2010), as described by Pot et al. (2021). The analyses were done upon clustering the bacterial and fungal ASV table with absolute sample counts at phylum, family, and genus level. Normalization based on the trimmed mean of M values was applied to correct for differences in library size of the count table. A design matrix was defined based on the experimental design, with a main effect for treatment. The dispersion parameter was calculated. Next, a negative binomial model was fitted for every ASV; then, the models of each ASV were combined into one general model. Likelihood-ratio tests were conducted on the contrast of the model parameters to assess differential abundances. P values < 0.05 were considered significant. Correction for multiple testing was included by adopting the Benjamini-Hochberg false discovery rate procedure. Fifth, the presence of beneficial microorganisms was studied, focusing on genera described to include biocontrol agents and that are often present in commercial biocontrol products, including *Trichoderma*, *Bacillus*, *Pseudomonas*, and *Streptomyces* spp. (Joos et al. 2020b; Lahlali et al. 2011; Law et al. 2017; Stockwell and Stack 2007) and biocontrol agents known to suppress *Phytophthora* spp. (i.e., *Trichoderma*, *Bacillus*, *Fusarium*, *Aspergillus*, *Penicillium*, *Streptomyces*, *Actinomadura*, and *Pseudomonas* spp.) (Duvenhage and Kotzé 1993; Macías-Rodríguez et al. 2018; Méndez-Bravo et al. 2018; Stirling et al. 1992; Turnbull et al. 1992; You et al. 1996). Sixth, correlations between relative abundances of genera and disease severity were determined using Spearman correlations. Finally, co-occurrence microbial networks of the different substrates were constructed by relative abundances of genera. Samples were grouped based on substrate, because there were too few replicates to distinguish between fertilization regimes, resulting in six samples per substrate. Correlations between genera were calculated using Spearman correlations. Networks were constructed with strong ($r > 0.75$) and significant ($P < 0.05$) correlations using the igraph package (version 1.2.11) (Csardi and Nepusz 2005). Topological properties of the networks were computed and visualised with gephi (version 0.9.2) (Bastian et al. 2009).

Differences in plant length were tested using a linear model that included substrate and fertilization regime as main effects and the interaction between them. General linear models were used to test differences in root development and disease severity, which included substrate and fertilization regime as main effects and the interaction between them. Linearity, homogeneity of variances, and normality were checked prior to analysis by plotting residuals versus fitted values, a QQ plot of the standardized residuals, and a scale-location plot.

RESULTS

Substrates based on management residues result in smaller plants but are disease suppressive depending on fertilization. Fertilization regime 2 resulted in significantly larger plants compared with fertilization regime 1 ($P < 0.001$). Both H and R resulted in significant smaller plants than the control substrate ($P < 0.001$ and $P < 0.001$, respectively) (Fig. 2A). No significant interaction effect was found between substrate and fertilization regime for plant length. Moreover, no significant effects of substrate or fertilization were found on root development.

For disease severity, a significant interaction effect of substrate and fertilization was found, by which the effect of substrate was evaluated within each fertilization regime. Overall, H1 showed a significantly lower disease severity than C1 ($P = 0.02$), while R2 showed a significantly lower disease severity than C2 ($P < 0.001$) (Fig. 2B).

Substrates based on management residues have more complex microbial networks in the rhizosphere than peat. Interactions in the rhizosphere microbiome of the different substrates were explored using microbial co-occurrence networks. Clear differences existed in the network topology of the different substrates, indicating that the microbial interactions were influenced by the substrate (Supplementary Table S3; Fig. 3). For bacteria, the microbiome of H and R formed a larger (with a greater number of nodes; 181 in H and 179 in R compared with 174 in C), more complex (with a higher number of edges; 36,540 in H and 29,026 in R compared with 17,856 in C), and more connected (with a larger average degree or node connectivity; 78.8 in H and 77.1 in R compared with 62.3 in C) co-occurrence network than the microbiome of substrate C. Larger clustering coefficients were observed in the bacterial community of H and R than in C, indicating closer relationships between nodes and their neighbours. H and R also exhibited larger modularity, indicating that the network is structured among densely connected groups of nodes. For fungi, the microbiome of H

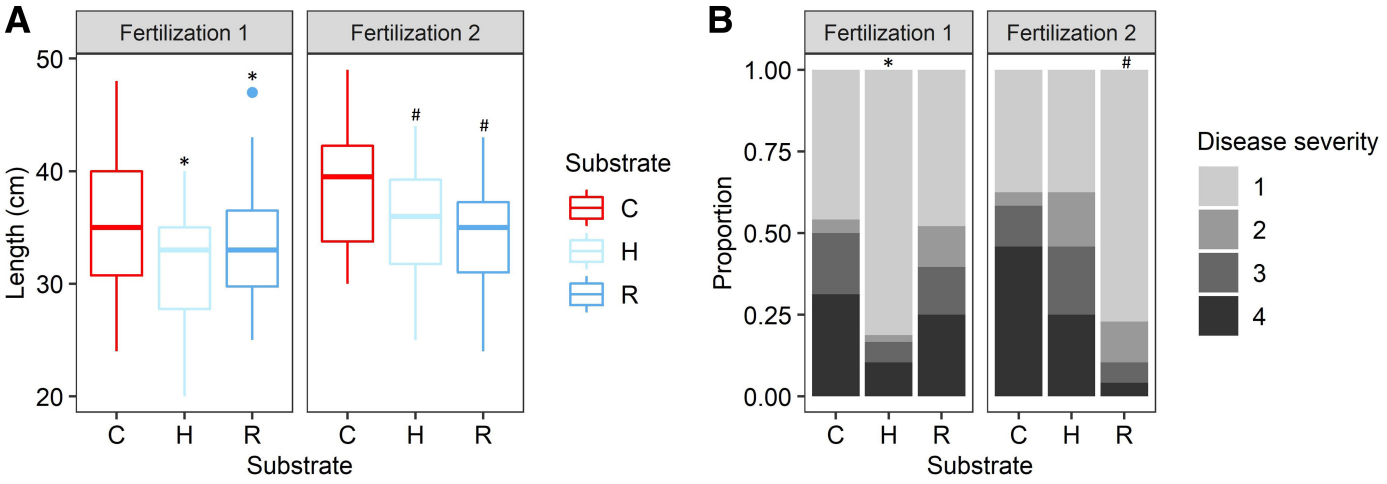

Fig. 2. A, Plant length in the different treatments. **B,** Disease severity (1 = no visible disease, 2 = small area of the plant visibly diseased, 3 = half of the plant visibly diseased, and 4 = whole plant visibly diseased) in the different treatments. An asterisk (*) indicates a significant difference compared with C1. A hash mark (#) indicates a significant difference compared with C2. C = control, H = chopped heath, R = soft rush, 1 = fertilizer regime 1, and 2 = fertilizer regime 2.

and R formed a smaller (with a smaller number of nodes; 154 in H and 127 in R compared with 156 in C) but more complex and more connected (with a larger number of edges; 8,804 in H and 10,205 in R compared with 5,496 in C; and a larger average degree; 46.8 in H and 38.3 in R compared with 30.9 in C) co-occurrence network than C. The fungal community of H and R showed a larger clustering coefficient, indicating closer connections between nodes and their neighbours. Smaller modularity was observed in the fungal community of H and R as compared with C. Although we observed differences between substrates in microbial networks, substrates H and R were also linked to lower disease severity (Supplementary Fig. S3). This may also affect the microbial interactions. However, it was not possible to detach substrate from disease severity in this dataset.

Bacterial and fungal diversity in the rhizosphere is not related to disease suppression. For bacterial and fungal diversity, a significant interaction was found between substrate and fertilization regime ($P = 0.009$). Overall, R2 showed a significantly lower bacterial and fungal diversity than C2 ($P = 0.004$ and $P = 0.005$, respectively) (Fig. 4), while no significant differences were found in bacterial and fungal diversity between H1, R1, and C1 or between H2 and C2. Thus, disease-suppressive treatments H1 and R2 do not show consistent differences in bacterial or fungal diversity compared with the references C1 and C2, respectively.

Disease-suppressive horticultural substrates show the largest shift in the rhizosphere community when compared with peat-based substrates. Differences in bacterial and fungal community composition were visualized by PCoA (Fig. 5). For bacteria, the first and second principal coordinates represented 32.6 and 24.3%, respectively, of the variance in the dataset whereas, for the fungal communities, these values were 37.4 and 28.5%, respectively. PERMANOVA showed a significant interaction between substrate and fertilization regime ($P = 0.002$ and $P = 0.001$) in the bacterial and fungal datasets, respectively. Within each fertilization regime, a significant effect of substrate was seen ($P = 0.006$ and $P = 0.008$, respectively, for the bacterial dataset, and $P = 0.005$ and $P = 0.003$, respectively, for the fungal dataset). The remark needs to be made that the condition of homogeneity of variances was not fulfilled

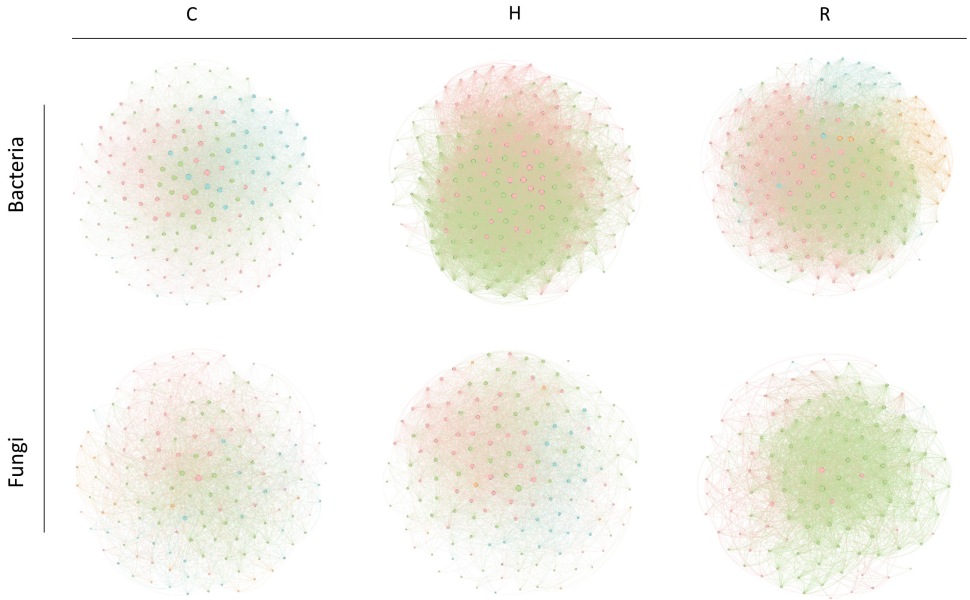

Fig. 3. Network of co-occurring bacterial and fungal genera in the different substrates: control substrate (C), heath chopper (H), and soft rush (R). A connection stands for a strong (Spearman $r > 0.75$) and significant (P value < 0.05) correlation. The size of each node is proportional to the degree (i.e., the number of connections). Colors indicate the different modules, representing groups of co-occurring microorganisms.

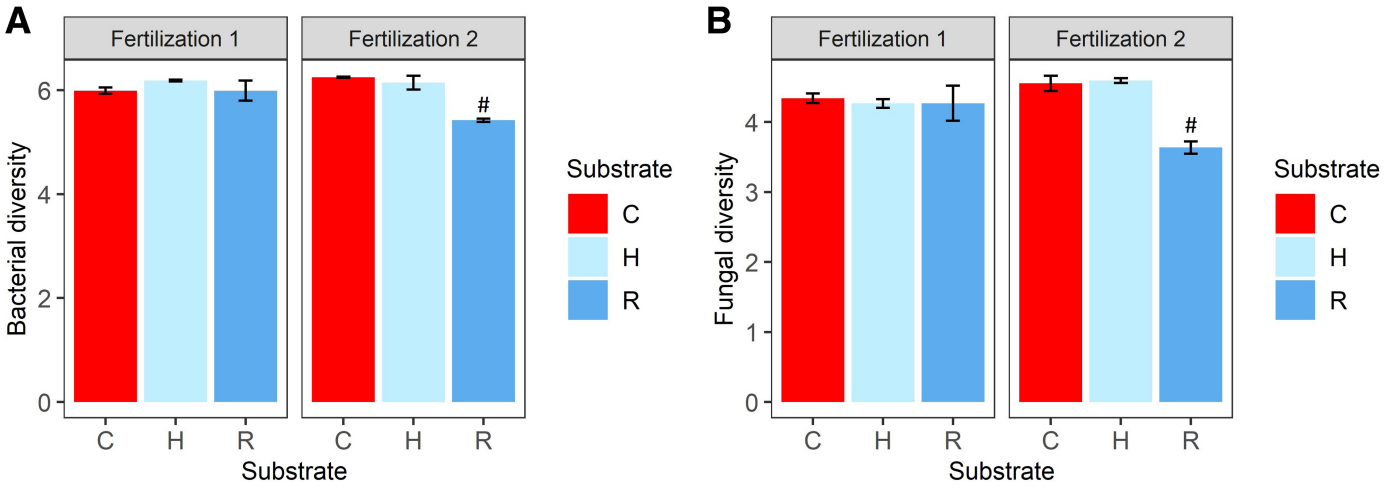

Fig. 4. A, Bacterial and **B,** fungal diversity expressed as the Shannon Diversity Index in the different treatments. A hash mark (#) indicates a significant difference compared with C2. C = control, H = chopped heath, R = soft rush, and 2 = fertilizer regime 2.

($P = 0.009$) in fertilization regime 2 in the bacterial dataset, indicating sample heterogeneity. Overall, fertilization had a large effect on the bacterial and fungal community composition in management residues but a relatively small effect in peat. Moreover, the largest difference in overall composition of bacterial and fungal community was observed between H1 and C1 and between R2 and C2. H1 and R2 also showed differences in their bacterial and fungal community composition.

The difference in bacterial and fungal community composition observed in the PCoA plots was studied in more detail by (i) looking into the most abundant bacterial phyla and genera, (ii) determining significantly associated genera, and (iii) determining differential abundances between C1, H1, and R1, and between C2, H2, and R2.

First, the most abundant ($n = 10$) bacterial (Supplementary Fig. S4) and fungal (Supplementary Fig. S5) taxa at genus level were studied. The top 10 most abundant bacterial and fungal genera in the two disease-suppressive treatments H1 and R2 showed the least similarity compared with C1 and C2, respectively. Moreover, the two disease-suppressive treatments H1 and R2 showed little similarity in the genera present in their most abundant bacterial and fungal genera and in the relative abundance of these genera (Supplementary Fig. S6).

Second, for each treatment, significantly associated genera (i.e., indicator genera) were determined (Supplementary Table S4). The genera known to include biocontrol agents, *Trichoderma* and *Aspergillus*, were significantly associated with H1. The genus *Bacillus*, known to include biocontrol agents, was significantly associated with R2.

Third, differential abundances of bacterial and fungal phyla, families, and genera were determined and showed significant differences between the different treatments (Table 1). The largest number of significantly differentially abundant bacterial and fungal genera was observed between R2 and C2. H1 showed a larger number of significantly differentially abundant bacterial and fungal genera compared with C1 than R1. Overviews of the significantly differentially abundant bacterial and fungal phyla, families, and genera between the different treatments are shown in Supplementary Tables S5 (phyla), S6 (families), and S7 (genera). Several genera were found to be significantly differentially abundant in both H1 as compared with C1 and R2 as compared with C2 (Supplementary Table S8; Supplementary Fig. S7).

Higher abundances of genera associated with biocontrol agents occur in disease-suppressive horticultural substrates. Genera that are associated with biocontrol agents for *Phytophthora* spp. were found in the different treatments and showed significant differences in their relative abundances (Fig. 6; Supplementary Fig. S8). The relative abundance of *Actinomadura* was significantly increased in R1 (6.90E-03 ± 3.72E-03) as compared with C1 (0.00E-00 ± 0.00E-00) and in R2 (9.86E-03 ± 2.12E-03) as compared with C2 (9.88E-05 ± 9.88E-05) (fold change [FC] = 100) and showed the highest relative abundance in R2. *Bacillus* was significantly more abundant in H1 (3.15E-04 ± 1.66E-04) and R1 (5.50E-03 ± 6.56E-04) compared with C1 (0.00E-00 ± 0.00E-00) and in R2 (1.33E-02 ± 2.80E-03) compared with C2 (7.06E-05 ± 7.06E-05) (FC = 189) and showed the highest relative abundance in R2. *Aspergillus* and *Trichoderma* were significantly more abundant in H1 (3.41E-03 ± 6.72E-04 and 1.28E-02 ± 2.41E-03, respectively) than in C1 (3.03E-04 ± 7.47E-05 and 1.33E-03 ± 3.45E-04, respectively) (FC = 11 and 10, respectively). *Pseudomonas* was significantly less abundant in R2 (0.00E-00 ± 0.00E-00) than in C2 (5.37E-04 ± 1.14E-04). *Streptomyces* was significantly less abundant in R1 (6.71E-03 ± 1.51E-03) than in C1 (1.99E-02 ± 7.23E-03) (FC = 0.34) and in R2 (4.40E-03 ± 4.34E-04) than in C2 (2.70E-02 ± 6.12E-03) (FC = 0.16). *Fusarium* was significantly less abundant in R2 (0.00E-00 ± 0.00E-00) than in C2 (1.07E-03 ± 3.36E-04).

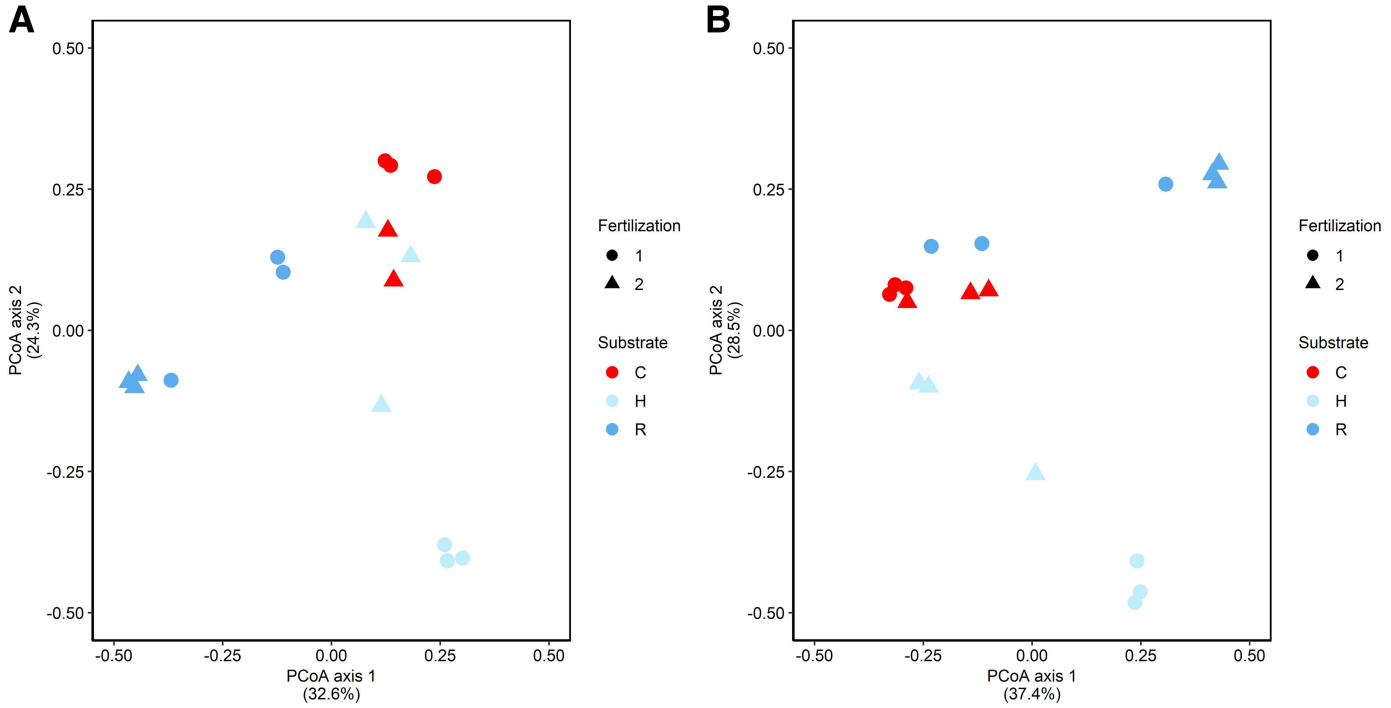

Fig. 5. Shifts in bacterial and fungal community composition between the different treatments. Principal coordinate analysis (PCoA) profile of pairwise community dissimilarity (Bray-Curtis) indices of **A,** bacterial (16S V3-V4 ribosomal RNA gene) and **B,** fungal (internal transcribed spacer 2 gene) sequencing data. Colors indicate the three types of substrates: control (C), chopped heath (H), and soft rush (R). Shapes show the two fertilization regimes: 1 = regime 1 and 2 = regime 2.

Genera of which the relative abundance was significantly correlated with disease severity were determined as well. The relative abundance of 48 bacterial genera and 63 fungal genera showed a significant correlation with disease severity (Supplementary Table S9). In all, 24 of these bacterial genera and 9 of these fungal genera showed a significant negative correlation with disease severity (Fig. 7). *Roseiarcus*, *Acidipila*, and *Jatrophihabitans* (bacterial) and *Chaetomium* and *Hypodiscus* (fungal) were the most abundant (>1%) genera that were significantly negatively correlated with disease severity in H1, while *Thermoactinomyces*, *Lacyella*, *Thermobispora*, *Geobacillus*, *Planifilum*, *Bacillus*, *Roseiarcus*, *Jatrophihabitans*, and *Thermopolyspora* (bacterial) and *Chaetomium*, *Chrysosporium*, *Thermomyces*, *Hypodiscus*, and *Sagenomella* (fungal) were the most abundant (>1%) genera that were significantly negatively correlated in R2. Several of these negatively correlated genera were differentially abundant in the different treatments (Supplementary Fig. S9). To determine causal relationships between the relative abundances of these genera and disease severity, additional experiments are required.

DISCUSSION

Management residues reduced growth as compared with peat but were disease suppressive depending on the fertilization regime. Because *C. lawsoniana* is an ornamental plant, length of the plant is an important characteristic for growers. However, the increase in

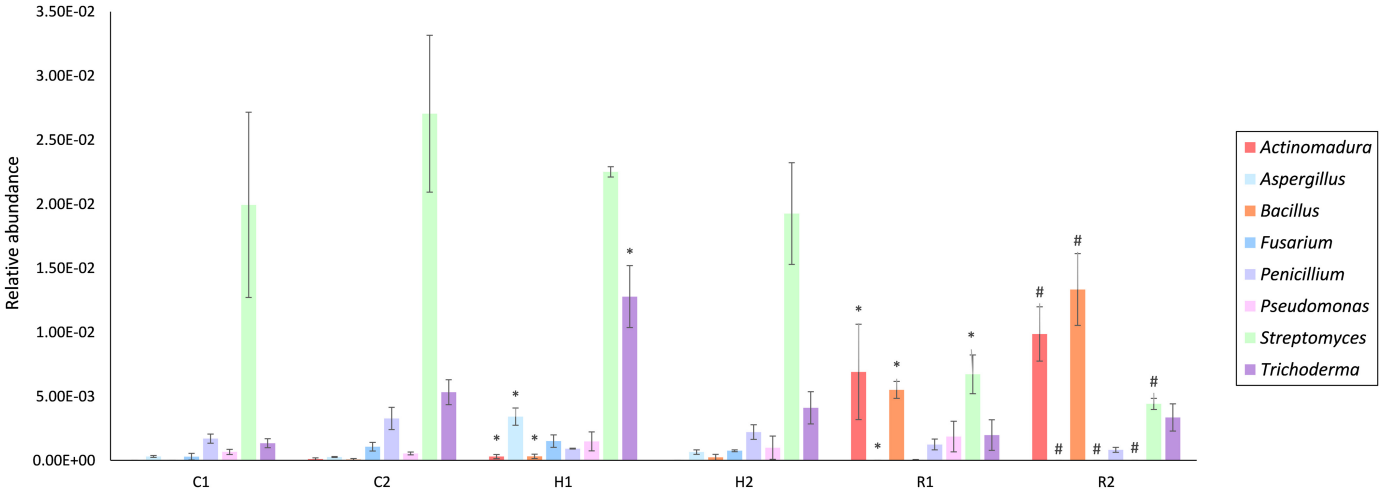

Fig. 6. Mean relative abundances ± standard error of genera that may include biocontrol agents for *Phytophthora* spp. An asterisk (*) indicates a significant difference in relative abundance as compared with C1 and a hash mark (#) indicates a significant difference in the relative abundance as compared with C2. C = control, H = chopped heath, R = soft rush, 1 = fertilization regime 1, and 2 = fertilization regime 2.

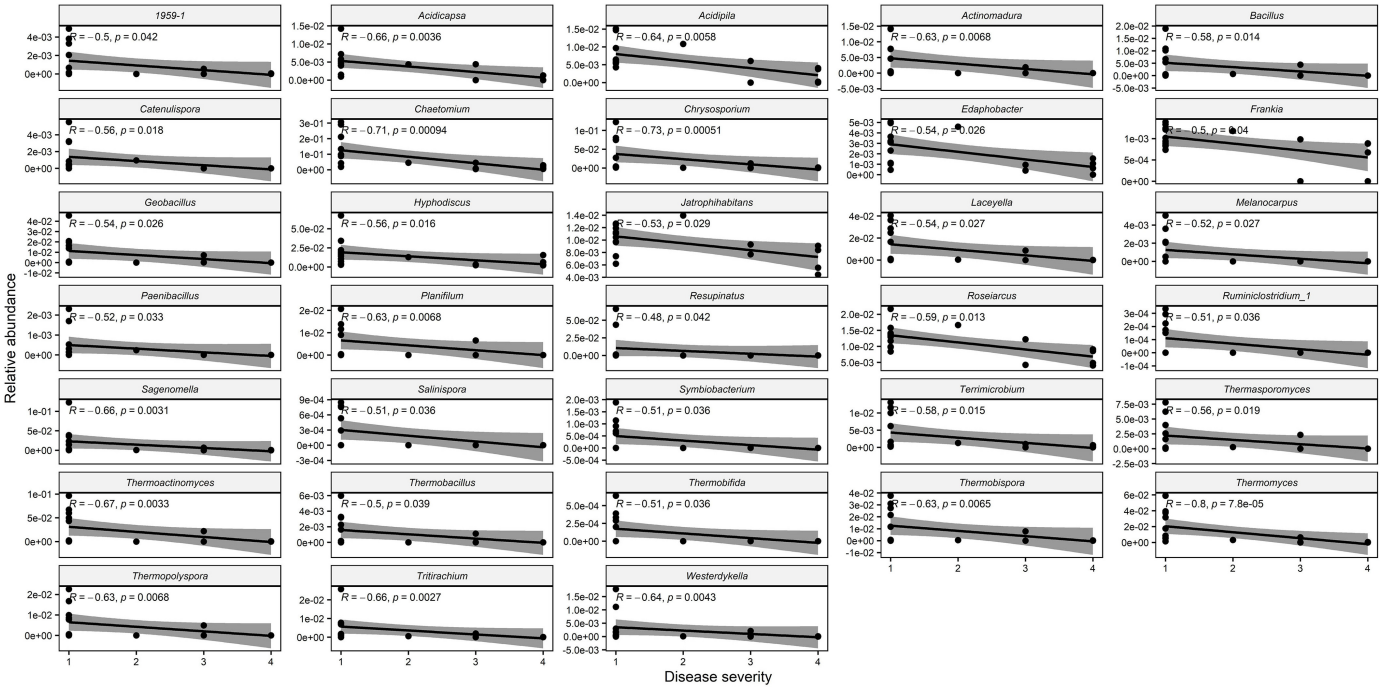

Fig. 7. Spearman correlations (*R*) between the relative abundance of significantly negatively correlated bacterial and fungal genera and disease severity. *P* values are shown.

disease suppression may compensate for smaller plants when using substrates based on management residues. In addition, the effect of management residues on growth was relatively small, with a reduction in length of only 8% in R and 10% in H, while disease severity was 38% lower in H with a pure N fertilizer and 48% lower in R with a compound fertilizer. The reason for the differences in growth may be related to differences in N availability in the substrates. Although an equal dose of N was supplied to all treatments, N availability in the pot may be affected by the blend. The observed reduction in growth in management residues may also be linked to lower levels of Ca and Mg in R and H. In comparison with composts, management residues contain lower concentrations of P, K, Ca, and Mg and, thus, have a lower potential for fertilizer replacement in horticultural substrates than composts. The effect of management residues on disease suppression was dependent on the fertilization regime. R in combination with a compound fertilizer and H in combination with a pure N fertilizer showed the highest disease suppression. Studies already showed that, for plant growth and quality, an interaction effect exists between substrate and fertilization (El-Naggar and El-Nasharty 2009; Mohamed 2018; Shalizi et al. 2019; Youssef 2014). However, to our knowledge, this is the first study to address the effect of sustainable horticultural substrates in combination with different fertilization regimes on disease suppression.

TABLE 1
Shifts in community distribution between the different treatments[a]

Reference	H1 C1	R1 C1	H2 C2	R2 C2
Bacterial taxa				
Phyla				
Significantly less abundant	8	1	0	8
Significantly more abundant	3	1	0	1
Total differentially abundant	11	2	0	9
Total not differentially abundant	10	19	21	12
Families				
Significantly less abundant	14	1	0	37
Significantly more abundant	21	15	0	18
Total differentially abundant	35	16	0	55
Total not differentially abundant	78	97	113	58
Genera				
Significantly less abundant	19	9	3	56
Significantly more abundant	25	27	2	26
Total differentially abundant	44	36	5	82
Total not differentially abundant	98	106	137	60
Fungal taxa				
Phyla				
Significantly less abundant	1	0	0	2
Significantly more abudant	3	1	0	0
Total differentially abundant	4	1	0	2
Total not differentially abundant	2	5	6	4
Families				
Significantly less abundant	19	3	2	28
Significantly more abudant	20	12	6	15
Total differentially abundant	39	15	8	43
Total not differentially abundant	51	75	82	47
Genera				
Significantly less abundant	40	4	0	42
Significantly more abudant	18	12	4	10
Total differentially abundant	58	16	4	52
Total not differentially abundant	65	107	119	71

[a] Total number of significantly altered bacterial and fungal taxa, number of significantly more abundant bacterial and fungal taxa, number of significantly less abundant bacterial and fungal taxa, and number of not differentially abundant bacterial and fungal taxa between the different treatments (H1, H2, R1, and R2) as compared with C1 and C2 at the phylum, family, and genus levels. C = control, H = chopped heath, R = soft rush, 1 = fertilization regime 1, and 2 = fertilization regime 2.

The observed disease suppression was not related to bacterial or fungal diversity in the rhizosphere. This is in contrast to what was expected based on literature (Bongiorno et al. 2019; Chaparro et al. 2012; Fliessbach et al. 2009; Jaiswal et al. 2017; Ou et al. 2019; van Elsas et al. 2012).

Fertilization had a strong influence on the community composition of the substrates with management residues but not so much on the communities of the peat-based substrates. Although the use of management residues in the substrates caused a shift in the community composition as compared with peat, the fertilization caused an additional shift in the management residues essential for disease suppression. Moreover, fertilization had different effects on the rhizosphere microbiome in the two types of management residues. The application of chemical fertilizers combined with organic amendments such as straw has been shown to have a different effect on the soil microbiome than the application of only chemical fertilizers (Li et al. 2020; Zhang et al. 2017). Zhang et al. (2017) showed that initial chemical properties of a soil such as pH influence the effect of chemical fertilizers on the soil microbiome. Thus, differences in the chemical properties of the substrates may explain the contrasting effects of fertilization on the rhizosphere microbiome.

A microbial community composition diverging from that of peat-based substrates seems most favourable in disease-suppressive horticultural substrates. Treatments that significantly decreased disease severity showed the largest shift in overall bacterial and fungal community composition compared with the peat-based substrates. This is in accordance with previous studies that have shown that peat is not a suitable medium for microorganisms and does not support biological control (Hoitink and Boehm 1999; Krause et al. 2001). Ou et al. (2019) also showed clear differences in bacterial and fungal community composition between conducive and suppressive soils.

The two disease-suppressive treatments showed clear differences in their bacterial and fungal community composition. That suggests that, although the microbial communities in these treatments have a different composition, their overall function is similar (Malacrinò et al. 2022). Doolittle and Booth (2017) showed that structurally different microbial communities may ensure the same ecosystem service.

Both the higher number of interactions in the microbial communities and the presence of beneficial microorganisms may contribute to disease suppression in the two disease-suppressive treatments. Network co-occurrence analysis revealed more complex interactions within the microbial communities in the substrates with H and R. The higher number of interaction in R and H suggests a greater degree of competition and predation (Deng et al. 2012). In addition, genera described to include biocontrol agents that may suppress *Phytophthora* spp. such as *Bacillus*, *Aspergillus*, *Actinomadura*, and *Trichoderma* spp. (Duvenhage and Kotzé 1993; Macías-Rodríguez et al. 2018; Méndez-Bravo et al. 2018; Stirling et al. 1992; Turnbull et al. 1992; You et al. 1996), showed the highest

abundances in the two disease-suppressive treatments. In H1, the highest relative abundances of *Aspergillus* and *Trichoderma* were observed and both genera were found as indicator genera whereas, in R2 *Actinomadura* and *Bacillus* showed the highest relative abundances and *Bacillus* was also found as an indicator genus. A trend of increasing relative abundances of beneficial genera was observed from the two management residue-based substrates that did not successfully suppress the pathogen (H2 and R1), to the two disease-suppressive treatments (H1 and R2). Moreover, the relative abundances of 24 bacterial and 9 fungal genera were negatively correlated with disease severity. Some of these genera such as *Bacillus, Actinomadura*, and *Chaetomium* are documented to have disease-suppressive capacities. For other genera, biocontrol properties have not been reported. Several of these negatively correlated genera, including *Bacillus*, *Chaetomium*, and *Actinomadura*, were significantly more abundant in at least one of the disease-suppressive treatments. The more complex interactions in the microbial communities, the high abundances of genera associated with beneficial microorganisms, and the presence of genera negatively correlated with disease severity in the disease-suppressive treatments may indicate the presence of both specific and general disease suppression. General disease suppression may be the result of activities of large, diverse, and active microbial communities. Competition and antibiosis are the main mechanisms involved in the suppressive effect. Specific disease suppression may be the result of antagonistic properties of specific beneficial microorganisms. Specific beneficial microorganisms most often induce disease suppression through mechanisms as predation, parasitism, or activation of disease resistance (Hadar and Papadopoulou 2012). Neher et al. (2022) stated that general disease suppression is most important in biological control of *Phytophthora* spp.

This study showed that disease suppressiveness in sustainable horticultural substrates is dependent on the fertilization regime and can be linked to changes in the microbial communities in the rhizosphere. Fertilization to optimize the nutrient balance in the peat-reduced horticultural substrates may also be used to shape the rhizosphere microbiome in management residues to increase disease suppression. Different types of management residues can be studied in further research to assess the potential of fertilizers to shape the rhizosphere microbiome and to further optimize disease suppression in horticultural substrates. To determine how fertilizers affect the rhizosphere microbiome and to optimize fertilization in terms of disease suppression in horticultural substrates, we suggest that further research should focus on adjusting composition and levels of fertilizers to assess the effects on the rhizosphere microbiome. In addition, the effect of organic fertilizers should be considered in further research. The present study showed that different microbial communities were associated with disease suppression. Although the composition of the rhizosphere microbiome can provide important information about the communities involved in disease suppression, the functions coded by these communities may provide additional information about their specific role in disease suppression (Malacrinò et al. 2022). Therefore, we suggest further research to focus on the specific mechanisms underlying disease suppression in horticultural substrates with management residues focussing on the metabolic profiles of the rhizosphere microbiome and the plant defence responses. Several bacterial and fungal genera were negatively correlated with disease suppression. Studies have shown that the composition of rhizosphere microbiomes in disease-suppressive soils can provide valuable information about which taxa may be used to improve plant health in other cultivations (Abdelfattah et al. 2018; Malacrinò et al. 2022; Trivedi et al. 2020). Therefore, in further research, it may be interesting to isolate strains, representative of these genera, from the disease-suppressive treatments and to inoculate them, either separately or as a consortium, to horticultural substrates in order to assess their effect on disease suppression.

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