Above- and belowground plant-associated microbiota interact to shape plant diversity-productivity relationships

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Abstract

Plant-associated microbes play a key role in mediating the relationship between plant diversity and productivity. However, previous studies have generally focused on a sole microbial guild (i.e. plant-beneficial microbes or pathogens), and on either aboveground or belowground microbes. As a result, the interplay among different microbial guilds and the overall impact of above- and belowground microbes on plant diversity-productivity relationships have rarely been investigated. Here we carried out an experiment where we applied microbial inocula collected from leaves and soils in the field onto plant leaves and soil in a greenhouse experiment with a herbaceous plant community. We showed that microbial inoculation of leaves reduced plant productivity and this negative effect was weaker at higher plant diversity, which promoted positive diversity-productivity relationships through complementarity effects. In contrast, microbial inoculation of soil alone had no impact on plant diversity-productivity relationships, but it counteracted the negative effects of leaf inoculum on plant productivity and weakened the leaf microbe-induced positive diversity-productivity relationships. We found that the abundance of arbuscular mycorrhizal fungi and Streptomyces bacteria increased when soil microbes were inoculated, and such increase was more significant at lower plant diversity, potentially explaining the effects of soil inoculation on plant productivity. These results suggest that the belowground plant beneficial microbes can counteract the effect of aboveground plant pathogens in mediating positive plant diversity-productivity relationships. Simultaneous study of plant-pathogenic and -beneficial microbes both above- and belowground is required to better understand the contributions of plant-associated microbes to biodiversity-ecosystem function relationships.

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We found that the abundance of arbuscular mycorrhizal fungi and*Streptomyces* bacteria increased when soil microbes were inoculated, and such increase was more significant at lower plant diversity, potentially explaining the effects of soil inoculation on plant productivity. These results suggest that the belowground plant beneficial microbes can counteract the effect of aboveground plant pathogens in mediating positive plant diversity-productivity relationships. Simultaneous study of plant-pathogenic and -beneficial microbes both above- and belowground is required to better understand the contributions of plant-associated microbes to biodiversity-ecosystem function relationships.

Key words

Biodiversity-productivity relationships, plant-associated microbes, plant pathogens, mycorrhizal fungi, below- and aboveground interaction

Introduction

Understanding the mechanisms that drive relationships between biodiversity and ecosystem function has been a major concern for ecologists and has attracted increasing attention given climate change and the loss of biodiversity . Studies to date have generally agreed that higher plant diversity contributes to increased ecosystem productivity through complementarity and selection processes , and that plant-associated microbes including plant pathogens and mycorrhizal fungi play a key role in mediating these processes . However, previous studies of the role of microbes in plant diversity-productivity relationships have generally focused on a single microbial guild of either aboveground or belowground plant microbiomes ; as a result, the interactions between microbial guilds above- and belowground and their collective effects on plant diversity-productivity relationships remain unknown. Theoretical studies have suggested that high complexity in trophic interactions could lead to varying relationships between biodiversity and ecosystem function, pointing out the necessity of considering complex plant associations with multiple microbial guilds both above- and belowground to understand the role of plant-associated microbes in determining the strength and direction of plant diversity-productivity relationships .

There is abundant empirical evidence that plant-associated microbes contribute to positive plant diversity-productivity relationships and several microbial functional groups have been implicated including plant pathogens such as foliar and soil-borne fungal pathogens, and plant-beneficial microbes including plant-growth promoting bacteria associated with leaves and roots as well as mycorrhizal fungi . The role of these microbial groups in shaping plant diversity-productivity relationships rests on a fundamental assumption that microbial impacts on plant productivity vary along gradients of plant diversity. For example, pathogens tend to reduce plant productivity at high plant conspecific density , and conspecific density is often negatively correlated with plant diversity, leading to the expectation that pathogenic effects should be weaker at higher plant diversity and thus that plant species should perform better in high-diversity communities compared to monocultures. The importance of pathogens for plant diversity and productivity has been well supported by the evidence that positive plant diversity-productivity relationships are weakened when plant pathogens are excluded and strengthened when pathogens are introduced in controlled experiments . Additionally, the diversity of plant-beneficial microbes has been observed to increase with plant diversity, which may increase the resource partitioning among plant species and promote positive diversity-productivity relationships through niche complementarity mechanisms . However, the role of plant-beneficial microbes such as arbuscular mycorrhizal fungi in shaping positive diversity-productivity relationships has not been widely supported by experimental evidence, presumably because mycorrhizal fungi are more host generalized than pathogens and their diversity and abundance do not covary with plant diversity significantly enough in experimental settings to lead to positive diversity-productivity relationships .

Plant-associated microbes can interact to determine the overall fitness of host plants, so that the impact of one microbial guild on plant diversity-productivity relationships may depend on the presence or absence of others . For instance, mycorrhizal associations improved host plant resistance during pathogen infection, leading to a more positive effect of mycorrhizal fungi on plant productivity when plant pathogens were present . Likewise, plant pathogens may cause greater reductions in productivity when host plants lack

mycorrhizal associations or have a low diversity of associated beneficial bacteria . Additionally, the interplay between microbial guilds can alter the way microbial effects change with plant diversity. For example, the high pathogen pressure at high host density (and hence low diversity) may trigger strong plant association with mycorrhizal fungi, which counteract the higher pathogenic effect at lower plant diversities . Previous studies that involved introduction or removal of whole microbial communities from leaves or roots have observed positive or negative effects on plant productivity, which were interpreted as either plant pathogens or beneficial microbes . However, it remains largely unexplored whether plant pathogens and beneficial microbes can interact to mediate plant productivity and diversity-productivity relationships (Jonsson *et al.* 2001; Ruijven *et al.* 2020).

Plant leaves and roots represent two major habitats for plant-associated microbes. Both above- and belowground plant parts harbor important plant pathogenic and beneficial microbes, while differing greatly in nutrient availability and other abiotic conditions . Many plant-associated microbes are found in association with both leaves and roots; for example almost half of the bacterial taxa in the phyllosphere of*Arabidopsis thaliana* were also found in the *A. thaliana*rhizosphere and soil . Additionally, the microbes in one habitat could influence the other by altering plant traits; for example, mycorrhizal associations belowground could improve plant nutrient uptake and modify plant traits such leaf chemistry which then influence the leaf microbial community . Both aboveground and belowground plant microbiomes can influence plant performance and thus may jointly mediate plant diversity-productivity relationships. However, studies to date generally have focused on either leaf- or root-associated microbes, and the interplay between plant leaf- and root-associated microbes and their collective effect on plant productivity are unclear.

To test if above- and belowground plant-associated microbes can interact to determine plant diversity-productivity relationships, we conducted a greenhouse experiment where we inoculated microbial communities collected from plant leaves and roots in the field onto plant communities of different species richness in the greenhouse. We predicted that the effect of above- and belowground microbial inoculation on plant productivity can change with plant diversity to influence plant diversity-productivity relationships. We also predicted that the effect of aboveground microbes on plant diversity-productivity relationships can be influenced by belowground microbes, resulting in a three-way interaction of plant diversity, above- and belowground microbes in determining plant productivity.

Materials and Methods

Experimental set-up

We created experimental plant assemblages composed of four native herbaceous species that commonly co-occur in grasslands at the Gault Nature Reserve, located on Mont Saint-Hilaire, Quebec, Canada. The reserve is comprised of mature temperate forests surrounding a 2-ha grassland. The herbaceous species *Solidago canadensis* ,*Asclepias syriaca* , *Achillea millefolium* and*Desmodium canadense* are the dominant species in this grassland habitat. The experimental plant assemblage consisted of 4 individuals of different species combinations of these four species, generating a total of 15 species compositions at 4 plant diversities: 4 monocultures, 6 two-species communities, 4 three-species community and a four-species community (Table S1). For each species composition, we imposed a soil treatment which applied either live or sterile soil microbe inoculum, and a leaf treatment which were high-concentration leaf microbe inoculum, low-concentration leaf microbe inoculum or sterile leaf microbe inoculum (Fig S1). Inoculation and sterilization details are described in the next paragraph. We established 4 replicates for each composition × treatment combination, resulting in a total of 1440 plant individuals in 360 plant communities (15 community compositions, 2 soil treatments, 3 leaf treatments and 4 replicates).

We grew these plant communities in greenhouse conditions beginning in November 2020. The seeds of the four species were purchased from a commercial company (Pepiniere Rustique, Quebec, Canada) and surface sterilized before use by treatment with 75% alcohol for 1 min and then washed using sterile water. Seeds were placed in propagation trays with Berger's BM6 classic peat-and-perlite growing medium (Berger, Quebec, Canada) to germinate for 10 days, and then transferred to multiple-cell plug insert trays with the same soil

substrate to establish for a week. After that, four seedlings were transplanted into a plastic pot (27 cm in diameter and 22 cm tall) containing approximately 4kg BM6 soil substrate to form a plant community; in total 360 pots were planted.

Microbial inoculation on plant roots and leaves

We collected microbial communities from plant roots and leaves in the Gault Nature Reserve in Summer 2020. We sampled 20 individuals of each of the four species used in the experiments. The roots of each individual were extracted from the soil and the soil adhering to the roots was collected. We obtained around 250g rhizosphere soil for each individual and thus 5 kg for each plant species. The soil from the different species and individuals were mixed thoroughly and preserved at -80°C for later use. For leaf-associated microbes, we collected approximately 150g of plant leaves from each individual into a sterile roll bag. We added 100ml sterile phosphate-buffered saline to the bag and agitated the leaves for 5 mins to wash off the microbes on the leaf surfaces. The resulting leaf wash solution was pooled across species, and then pelleted for 10 mins at 3000g. We carefully transferred the pellets into 25% glycerol freezing buffer and stored the extracted microbes at -80°C for later use.

We inoculated the soil and leaf microbial inocula onto plant communities in pots in the greenhouse one week after plants established. For soil microbe inoculation, we thawed the soil-derived inoculum and then divided it into two equal parts. One part was used for a microbial soil inoculum and the other part was sterilized in an autoclave for 30 minutes at 121°C used as a control. We added 50g soil of either microbial inoculum or control into each pot and mixed it with the background soil substrate. For leaf microbe inoculation, we thawed the leaf-derived inoculum in glycerol buffer and centrifuged the cells at 3000g for 10 mins. The pellets were collected, resuspended in 300ml 10mM $MgCl_2$ buffer and diluted to 3L using the same buffer. This was then equally divided into 3 parts, with one part sterilized in an autoclave for 30 mins at 121°C as the control, one part diluted by 100 times as a low-concentration microbial inoculum, and the remaining part used as a high-concentration inoculum. We sprayed approximately 6-8 ml of inoculum onto the aboveground plant surfaces in each pot. Immediately after inoculation, we increased the humidity of the greenhouse to 90% for 24 hours to provide a high humidity misting condition for microbes to establish on leaves . We performed a second leaf microbe inoculation one week after the first inoculation following the same procedure as above to ensure a high probability of colonization of leaves by the microbes in the inoculum.

After microbial inoculation treatments, the pots were arranged randomly with a 20-cm spacing between pots to avoid contact between leaves and to reduce the chance of contamination across treatments, and the position of pots was randomly shifted every two months. We grew plants in the UQAM greenhouse in Montreal, Canada at 24°C and 50% relative humidity. After growing for 20 weeks, we harvested and oven-dried the plants at 60°C for 48 hours, and then weighed the plants to determine the biomass of each plant individual. Meanwhile, we collected 2.5g soil from a random replicate of each of our treatments (90 samples, i.e. 15 plant community treatments and 2 soil and 3 leaf treatments) to identity the soil microbial taxa at the end of our experiments.

Soil microbial community composition

We extracted microbial DNA from the 90 soil samples using PowerSoil Pro DNA extraction kits (QIAGEN) following the manufacturer's protocol. However, we were unable to collect leaf microbes at the end of the experiment because washing leaves of herbaceous species often damages plant tissues which could bias estimates of plant biomass. We also extracted microbial DNA from leaf and soil inoculum collected in the field as well as background soil substrate filled in the pots at the beginning of the experiment.

We amplified the ITS2 rDNA region to determine the composition of fungal communities using a two-step nested PCR. The first step was conducted using a mixture of SSUmAf (SSUmAf1 and SSUmAf2) as forward primers and a mixture of LASmAr (LASmAr1-4) as reverse primers. The use of this primer set allowed us to amplify *Glomeromycota* in order to enhance the identification of arbuscular mycorrhizal fungi, which is a major beneficial microbial group associated with herbaceous plants . We prepared triplicate 25 μL PCR reagent with 5 μL 5× Phusion HF Buffer (Thermo Fisher Scientific: Frederick, Maryland, USA), 0.5 μL

dNTPs (10 mM), 0.75 μL DMSO, 0.5 μL primer mix (10 μM each primer, 2 forward and 4 reverse primers), 0.25 μL Phusion Hot Start II polymerase (2 U/μL) (Thermo Fisher Scientific), 2 μL DNA template and 15.5 μL molecular-grade H2O. PCR reactions were performed using the following condition: 30 s initial denaturation at 98°C, followed by 40 cycles of 10 s at 98°C, 30 s at 60°C, and 1 min at 72°C, with a final 10-minute elongation at 72°C. After that, we conducted the second step PCR using ITS7o and ITS4 as forward and reverse primers respectively . The PCR reagent was prepared with the same components as the first step and running with the following condition: 30 s initial denaturation at 98°C, followed by 31 cycles of 10 s at 98°C, 30 s at 49°C, and 30 s at 72°C, with a final 10-minute elongation at 72°C.

The V5-V6 region of bacteria 16S rRNA genes was amplified using primers 799F and 1115R to determine the composition of bacterial communities. We used the same PCR reagents as described above and ran the PCR with the following conditions: 30 s initial denaturation at 98°C, followed by 35 cycles of 15 s at 98°C, 30 s at 64°C, and 30 s at 72°C, with a final 10-minute elongation at 72°C. The PCR products of both ITS2 and 16S amplicons were normalized using a SequalPrep Normalization kit (Thermo Fisher Scientific), then pooled and purified using AMPure (Beckman Coulter Life Sciences: Brea, California, USA) to avoid contaminants. After that, we prepared the DNA library by mixing equimolar concentrations of DNA for each sample and sequenced the DNA on an Illumina MiSeq using Illumina MiSeq reagent kit v3 (Illumina: Hayward, California, USA)

Analysis

We calculated productivity as the aboveground plant biomass for each plant community. A small proportion (˜4%) of plants died during the experiment, of which almost all (˜94%) were *Asclepias syriaca* . To determine if the death of plants was influenced by inoculation treatments, we modeled the survival of*A. syriaca* as a function of soil and leaf microbe inoculation. We found no effect of inoculation treatments on survival. We excluded the plant communities with dead individuals, leaving 316 out of 360 plant communities that were included in further analyses. During the sampling, we found a different phenotype of *Solidago canadensis* in 5 of out the 316 pots, the analysis with and without the 5 pots show consistent results. Therefore, we present here the results of analyses including these phenotypically distinct individuals.

We used linear mixed-effect models and analysis of variance (ANOVA) (packages 'lme4' and 'lmeTest' in R version 4.2.0;) to test effects of plant diversity, soil microbial inoculation, leaf microbial inoculation and all interactions among these three explanatory variables on plant productivity. To control the effect of plant community composition on plant productivity, we included the composition as a random term in the model . To specifically test the effect of microbial inoculations on plant productivity and plant diversity-productivity relationships, we contrasted the marginal means of plant productivity between microbial treatments using Tukey's HSD post hoc test; and compared the slopes of diversity-productivity relationships between microbial treatments using the *emtrend* function in the 'emmeans' package (Lenth et al. 2018). We further calculated net biodiversity effects, also known as overyielding, for all pots with more than two plant species and partitioned the biodiversity effect into selection and complementarity components according to Loreau and Hector (2001). We tested the effect of microbial inoculations on net biodiversity effects, selection effects, and complementarity effects using Tukey's HSD post hoc test. Since we did not observe any significant effect of low-concentration leaf microbial inoculum on productivity, we only present comparisons regarding high-concentration microbial inoculum when discussing leaf microbial effect although all data were included in our analysis.

We used DADA2 to identify the amplicon sequence variants (ASV) of bacteria and fungi . For bacterial 16S sequences, we calculated the quality scores (Q) of the reads to inspect the sequencing quality, then we removed 30 and 28 nucleotides from the start of reads and kept total sequence length at 180 and 220 for forward and reverse sequences respectively. This trim method removed the primers and low-quality nucleotides resulting in reads of fixed length. However, the ITS2 region of fungi is highly variable in length and this variation reflects the biological differences between fungal taxonomic groups. Thus, we only removed the primers from the ITS amplicon sequences but did not trim them to fixed length. After that, we inferred the error rates of nucleotide substitution and assigned sequences to ASVs following DADA2 default parameters . The paired

read ends were merged with a minimum overlap of 12 nucleotides, and non-target sequences and chimeras were removed. Bacterial and fungal taxonomy was assigned by comparison with the SILVA SSU r138 and ITS v8.3 databases respectively .

The total number of reads of both bacterial and fungal ASVs varied greatly among samples which could bias the estimation of sample diversity. Thus, we rarefied the community data using the 'vegan' R package (Oksanen, J., et al. 2022). For soil microbial communities collected at the end of the experiment, we firstly used generalized linear models (Poisson family with log link function) to test for effects of plant diversity, soil and leaf treatments on microbial ASV richness (alpha diversity) and then applied distance-based redundancy analysis on the Euclidean distance matrix of Hellinger-transformed community data to test the effects of these treatments on microbial community composition (beta diversity). Additionally, we used Permutational multivariate analysis of variance (PERMANOVA) to quantify the variation in microbial community composition explained by plant diversity, soil, and leaf inoculation respectively. We did not consider the interactions among plant diversity, soil and leaf inoculation in microbial analysis due to a limited number of microbial samples. Finally, we conducted ANCOM-BC analysis to identify the microbial taxa with differential abundance between inoculated and non-inoculated soils using the 'ANCOMBC' R package .

Results

The effect of microbial inoculation on plant productivity

Inoculation of leaf microbial inoculum reduced plant productivity while inoculation of soil microbial inoculum increased plant productivity (Table 1, Fig. 1). There was a significant interaction between soil and leaf microbial inoculum in affecting plant productivity (Table 1, $F_{(2,\ 292)} = 7.34$, $p < 0.001$). Specifically, the negative effects of high-concentration leaf microbial inoculum on plant productivity became weak when soil microbes were inoculated, e.g. high-concentration leaf microbial inoculation decreased plant productivity by 27% with no soil inoculation but decreased productivity by only 3% with soil microbe inoculation (Fig. 1a). Conversely, the positive effect of soil microbes on productivity was only significant when leaf microbes were inoculated, e.g. soil microbe inoculation increased productivity by 26% when leaf inoculum was applied while decreased plant productivity by 5% when soil microbes were inoculated alone (Fig. 1b).

Microbial inoculation alters plant diversity-productivity relationships.

There was a significant interaction between plant diversity and soil inoculation effects on plant productivity (Table 1, $F_{(1,\ 291)} = 14.33$, $p < 0.001$), and a marginally significant effect of the interaction between plant diversity and leaf inoculation ($F_{(2,\ 291)} = 2.63$, $p = 0.07$), suggesting that soil inoculation changed the relationships between plant diversity and productivity. There was also a significant effect of the three-way interaction of plant diversity, soil and leaf microbe inoculation on plant productivity (Table 1, $F_{(2,\ 291)} = 3.99$, $p = 0.02$), suggesting that the effect of one inoculum on diversity-productivity relationships depended on the other inoculum. Specifically, inoculation of leaf inoculum decreased plant productivity at low diversity which increased the slope of positive plant diversity-productivity relationships, however, the effect of leaf inoculation disappeared when soil microbes were inoculated. In contrast, soil inoculation alone had no significant effect on plant diversity-productivity relationships, while on the condition of leaf microbes inoculated (i.e. comparing 'both soil and leaf inoculation' versus 'leaf-inoculation'), soil inoculation increased plant productivity at low diversity and thus weakened the positive diversity-productivity relationship (Table 2, Fig. 2).

The net biodiversity effect of diversity on plant biomass significantly differed among microbial inoculation treatments. Compared to non-microbial inoculation, the net biodiversity effect increased with leaf microbes inoculated and decreased with soil microbes inoculated, and inoculation of both leaf and soil microbes lead to a reduced biodiversity effect on plant biomass. The contribution of leaf microbial inoculation to net biodiversity effect was through increasing complementarity among species rather than a selection effect (Fig. 3).

Effects of inoculation on soil microbial communities

A total of 506 fungal ASVs and 4808 bacterial ASVs were identified from the soil samples collected at the

end of the experiment.*Chytridiomycota* and *Basidiomycota* were the most abundant fungal phyla, accounting for 54.0% and 19.7% of the total sequence abundance. The relative abundance of *Chytridiomycota* increased while that of *Basidiomycota* decreased in microbe-inoculated soils versus in non-inoculated soils (Fig. 4a). The arbuscular mycorrhizal fungal group *Glomeromycota* was the fourth most abundant phylum with an averaged relative abundance of 8.1% and they were found exclusively in microbe-inoculated soil. We detected *Mucoromycota*in leaf inoculum and *Glomeromycota* in soil inoculum at the beginning of the experiment, which may be the source of fungi in soil samples collected at the end of experiment. The soil bacterial community was dominated by *Actinobacteriota* and *Proteobacteria* , accounting for 68.6% and 17.7% of total sequence abundance respectively. The relative abundance of these two phyla was consistent in all soil samples, soil inoculum and the background soil substrate (Fig. 4b).

Inoculation of soil microbes increased soil fungal ASV richness but had no effect on bacterial ASV richness, in contrast, inoculation of leaf microbes decreased soil bacteria ASV richness but showed no influence on fungal ASV richness (Table 3). We also found plant diversity increased the richness of bacterial but not fungal ASVs (Table 3). Both soil inoculation treatment and plant diversity influenced microbial community composition; distance-based redundancy analysis showed significant differences in microbial community composition along plant diversity gradients and between soil inoculation and non-inoculation treatments (Fig. 5), and PERMANOVAs showed that plant diversity and soil inoculation treatment respectively explained 1.7% and 9.6% of the variation in fungal community composition and 2.4% and 12% of the variation in bacterial community composition (Table 4).

We identified 30 bacterial ASVs and 4 fungal ASVs that were significantly more abundant in inoculated soils versus non-inoculated soils. Most of the differentially abundant ASVs belonged to the bacterial genus *Streptomyces* and fungal genus*Spizellomyces* (Fig. S2). These taxa along with arbuscular mycorrhizal fungi which were only found in inoculated soils were potentially the causes of soil inoculation effect, leading us to analyzing the diversity and abundance of these particular taxonomic groups. We found that both the richness and abundance of arbuscular mycorrhizal fungi increased when high-concentration leaf microbe inoculum was inoculated and decreased with plant diversity (Table S2), and the richness of *Streptomyces* decreased with plant diversity (Table S2).

Discussion

Plant interactions with microbes are a key mechanism that shapes the relationship between plant diversity and productivity . Our results further illustrate that plant leaf- and root-associated microbes including plant-beneficial microbes and pathogens can interact to determine the strength of plant diversity-productivity relationships. Leaf microbial inoculation reduced plant productivity potentially due to leaf pathogen infection, which is stronger in lower-diversity plant assemblages leading to positive plant diversity-productivity relationships. However, this leaf microbe-induced positive diversity-productive relationship was weakened when soil microbes including arbuscular mycorrhizal fungi were inoculated, suggesting that the role of aboveground plant pathogens in mediating positive diversity-productivity relationships can be compromised by plant-beneficial microbes belowground. The interaction between leaf- and root-associated microbes and between plant pathogens and beneficial microbes has complicated our understanding of the influence of microbes on ecosystem productivity, highlighting the importance of considering both above- and belowground microbes and their collective effects in shaping plant diversity-productivity relationships.

Plant pathogens have been proposed as a major determinant of positive plant diversity-productivity relationships, based on the assumption that natural enemies decrease plant productivity at high conspecific density (low diversity) which consequently contributes to a positive plant diversity-productivity relationship . However, previous studies have generally focused on soil-borne fungal pathogens (but see Huang et al. 2022). In our study, we collected leaf microbial communities from plants growing in the field, where we observed obvious symptoms of plant disease caused by plant pathogens such as rust fungi. Inoculation of this microbial community onto plant leaves significantly decreased plant productivity at low plant diversity but barely at high diversity, resulting in positive diversity-productivity relationships. The result is consistent with a recent study conducted in a large subtropical forest biodiversity-ecosystem function experiment, where the

application of fungicide on tree canopies eliminated positive tree species diversity-productivity relationships . Additionally, we showed that inoculation of leaf microbes increased the overyielding of plant biomass (a net biodiversity effect on plant productivity), and this overyielding results from complementarity among plant species rather than a selection effect caused by a particular species. Indeed, the pathogenic effect can be 'diluted' by plant diversity because high-diversity plant assemblages often have low conspecific density. This diluted pathogenic effect allows plant species to perform better in mixed communities than monoculture, which induced interspecific complementarity and promote positive diversity-productivity relationships .

In contrast with plant pathogens, beneficial microbes such as mycorrhizal fungi have been suggested to promote plant productivity at high plant diversity and thus contribute to positive diversity-productivity relationships . This hypothesis was based on the observation that the diversity of mycorrhizal fungi increased with plant diversity . However, our experiment showed contrary results. Inoculation of belowground microbes including mycorrhizal fungi had no effect on plant diversity-productivity relationships, but when leaf inoculation was present, these belowground microbes showed beneficial effect on plant productivity especially at lower plant diversity, thereby weakening the positive diversity-productivity relationship. This result suggested that beneficial microbes can mediate diversity-productivity relationship though their interaction with plant pathogens. This observation is consistent with the findings of previous studies that strong plant-mycorrhizal associations were triggered at high pathogen pressure and promoted plant growth by defense against pathogen infection . Therefore, our findings implied alternative roles of beneficial microbes in mediating plant diversity-productivity relationship, that is, beneficial microbes may counteract pathogenic effect at low plant diversity, and thus weaken diversity-productivity relationships .

Although our findings demonstrated a significant interplay between aboveground plant pathogens and belowground beneficial microbes in shaping plant diversity-productivity relationships, it is important to acknowledge that our results are based on an experiment in greenhouse conditions. Whether these microbial effects observed in greenhouse conditions reflect those within natural ecosystems is not clear. Previous studies that test plant diversity effects on productivity often manipulate plant diversity in field conditions, but microbial transplants in the field can be challenging due to the random dispersal of microbes through air and rain. Future studies should extend our experiment to the field and could additionally manipulate microbial diversity by using fungicide and bactericide in addition to inoculation with microbes. Additionally, our experiment was limited in the number of plant species included, due to the need to consider several different microbial treatments; including more plant species would have greatly increased the resources required for this experiment, but as a result the relatively small plant diversity gradient we examined may restrict the broad applicability of our findings to more diverse ecosystems. Despite these caveats, our results clearly demonstrated the interaction between above- and belowground microbes in shaping plant diversity-productivity relationships, which can serve as a starting point for investigating how different microbial groups can interact to drive ecosystem functioning.

Changes in the diversity and composition of microbial communities along plant diversity gradients is the proposed mechanism for plant-associated microbes mediating diversity-productivity relationships . Our study presented evidence that bacterial and fungal community composition in soils changed with plant diversity, suggesting both soil fungi and bacteria can play a role in affecting plant diversity-productivity relationships . Particularly, the diversity and abundance of arbuscular mycorrhizal fungi and potentially plant growth-promoting*Streptomyces* bacteria decreased with plant diversity, which may explain the stronger positive effects of soil inoculation on plant productivity at lower plant diversity . Additionally, the diversity and abundance of arbuscular mycorrhizal fungi increased when leaf microbes were inoculated, supporting the idea that the beneficial effect of soil microbes can be triggered by potential pathogens on leaves . Identifying the microbial taxa that drive diversity-productivity relationships and characterizing how above- and belowground microbial communities change along gradients of plant diversity will be essential for understanding the influence of microbiomes on plant diversity-productivity relationships . However, without microbial isolation and manipulation, it's not certain whether these microbial groups caused the observed inoculation effect in our study. It would be intriguing for future studies to isolate the major pathogens and beneficial microbes on leaves and roots and inoculate these specific groups onto plant communities to test their respective and

joint effect on plant productivity.

By inoculating the entire microbial communities collected from plant rhizosphere soil and leaves in the field onto newly germinated plants in a greenhouse experiment, we demonstrated an overall negative effect of plant leaf inoculum on plant productivity, implying a net pathogenic effect of leaf microbes that promoted positive plant diversity-productivity relationships. However, when soil microbes were inoculated, this pathogenic effect of leaf inoculum was reduced, and the positive plant diversity-productivity relationship was weakened. These findings provide evidence for the interaction between aboveground microbes and belowground microbes in shaping plant diversity-productivity relationships in a greenhouse setting, which paves the way for studying the role of different plant-associated microbial groups and their interplay in mediating biodiversity-ecosystem functioning relationships in the field and in more diverse ecosystems.

Reference

Tables

Table 1 ANOVA table summarizing the result of a linear mixed-effect model where plant biomass was modeled as a function of plant diversity, soil inoculation, leaf inoculation and their interactions with plant community composition as a random term. Plant diversity was treated as a numeric variable to show whether higher plant diversity promote plant productivity (*p < 0.05; **p < 0.01; ***p < 0.001)

	Sum_Sq	Mean_sq	NumDF	DenDF	F_val	Pr(>F)	
Plant diversity	11.93	11.93	1	13	1.58	0.23	
Soil inoculation (Soil)	97.5	97.5	1	292	12.91	<0.001	***
Leaf inoculation (Leaf)	57.87	28.93	2	291	3.83	0.02	*
Diversity:Soil	108.22	108.22	1	291	14.33	<0.001	***
Diversity:Leaf	39.72	19.85	2	291	2.54	0.07	.
Soil:Leaf	110.8	55.42	2	292	7.34	<0.001	***
Diversity:Soil:Leaf	60.25	30.13	2	292	3.99	0.02	*

Table 2 Pairwise comparisons of marginal means of plant biomass between microbial treatments across plant diversities. We built a linear mixed-effect model of plant biomass predicted by plant diversity, leaf inoculation, soil inoculation and their interactions with plant composition as a random term. Plant diversity was treated as a character variable in the model to show how plant biomass differ among different diversity levels. The marginal means of plant biomass were extracted from the model and contrast between microbial treatments for each diversity level using T-test. The estimates of pairwise contrasts were reported where positive/negative values indicates positive/negative effect of microbial inoculation on plant biomass (see Table S5 for the statistics, *p < 0.05; **p < 0.01; ***p < 0.001).

		Plant diversity	Plant diversity	Plan
Comparisons	Conditions	1	2	3
Effect of Leaf inoculation (versus non-leaf inoculation)	With soil inoculation	0.83	-1.07	-0.86
	Without soil inoculation	-3.46 ***	-2.42 *	-0.75
Effect of soil inoculation (versus non-soil inoculation)	With leaf inoculation	5.51 ***	0.84	-0.80
	Without leaf inoculation	1.22	-0.50	-0.70

Table 3 Summary of generalized linear models that modeled microbial ASV richness as a function of plant diversity, soil and leaf inoculation treatments. The models were fitted separately for soil fungi and bacteria. Abbreviation: Soil, soil inoculation; Leaf1, low-concentration leaf microbial inoculation and Leaf2, high-concentration leaf microbial inoculation (*p < 0.05; **p < 0.01; ***p < 0.001).

	Estimate	Std.Error	Z values	Pr(>|z|)	
Fungal richness					
(Intercept)	2.55	0.08	30.34	<0.001	***
Plant diversity	-0.01	0.03	-0.19	0.85	
Soil	0.54	0.05	10.32	<0.001	***
Leaf1	-0.02	0.06	-0.36	0.72	
Leaf2	0.05	0.06	0.74	0.46	
Bacterial richness					
(Intercept)	6.43	0.01	479.92	<0.001	***
Plant diversity	0.01	0.00	2.45	0.01	*
Soil	0.00	0.01	-0.13	0.90	
Leaf1	-0.04	0.01	-3.48	<0.001	***
Leaf2	-0.04	0.01	-4.29	<0.001	***

Table 4 Summary of PERMANOVAs analyzing the variation in fungal and bacterial community composition explained by plant diversity, soil and leaf microbial inoculation (*p < 0.05; **p < 0.01; ***p < 0.001).

	Df	SumOfSqs	R^2	F	Pr(>F)	
Fungi						
Plant diversity	1	1.20	0.016	1.62	0.02	*
Soil inoculation	1	7.01	0.096	9.46	<0.001	***
Leaf inoculation	2	1.52	0.021	1.03	0.38	
Residual	85	63.02	0.86			
Total	89	72.76	1			
Bacteria						
Plant diversity	1	0.83	0.024	2.39	0.01	*
Soil inoculation	1	4.23	0.12	12.23	<0.001	***
Leaf inoculation	2	0.55	0.015	0.80	0.73	
Residual	85	29.46	0.83			
Total	89	35.09	1			

Figure captions

Fig. 1 The effect of (a) leaf- and (b) soil-microbial inoculation on plant productivity. Plant biomass was fitted in a linear mixed-effect model as a function of plant diversity, soil, leaf inoculation and their interactions with plant community composition taken as a random term. From the model the marginal means of plant biomass were extracted and contrasted between microbial treatments (see Table S3 for the statistics, *p < 0.05; **p < 0.01; ***p < 0.001). The mean value and standard error of plant biomass were shown.

Fig. 2 The relationships between plant diversity and biomass for different microbial inoculation treatments. A linear mixed-effect model was fitted to predict plant biomass as a function of plant diversity, leaf and soil inoculation and their interactions with plant composition taken as a random effect. The slopes of plant diversity-biomass relationships were extracted from the model and compared among microbe treatments using Tukey's HSD, with different letter labels in the box indicating significant difference in the diversity-biomass slopes. Standard errors of plant biomass were shown. We only present high-concentration leaf microbial inoculum since low-concentration inoculum has no effect on plant productivity (See Method).

Fig. 3 The net biodiversity effect (a), complementarity (b) and selection effect(c) of plant biomass across microbial inoculation treatments. The difference of biodiversity effect between groups was tested using

Tukey's HSD. Groups with different letter labels above the boxes have significantly different biodiversity effect at $p<0.05$.

Fig. 4 The phylum-level taxonomic composition of (a) fungi and (b) bacteria in microbial samples. Non-inoculated and inoculated soils were collected at the end of the experiment, with L.0, L.1 and L.2 represent non-leaf microbe treatment, low- and high-concentration leaf microbe treatments respectively. Leaf and soil inoculum as well as background soil were collected at the beginning of the experiment, representing the leaf and soil microbial communities collected in the field and the background soil substrate used in the study.

Fig. 5 Distance-based redundancy analysis indicating the variation in (a) bacterial and (b) fungal soil community composition across microbial inoculation treatments and plant diversity. Arrows represent the effect of plant diversity, soil inoculation (Soil), low-concentration leaf inoculation (Leaf1) and high-concentration leaf inoculation (Leaf2) on microbial community composition, with significant relationships indicated by asterisks (see Table S4 for the statistics, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Figures

Fig. 1

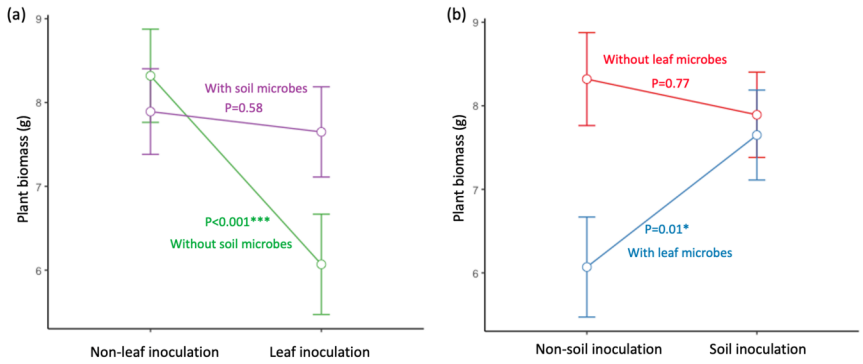

Fig. 2

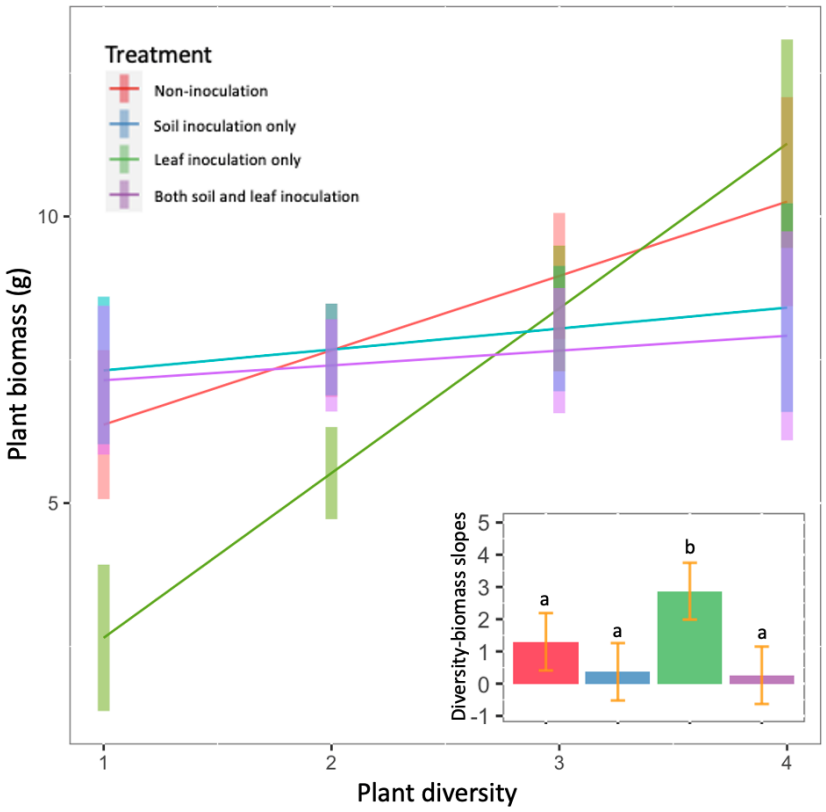

Fig. 3

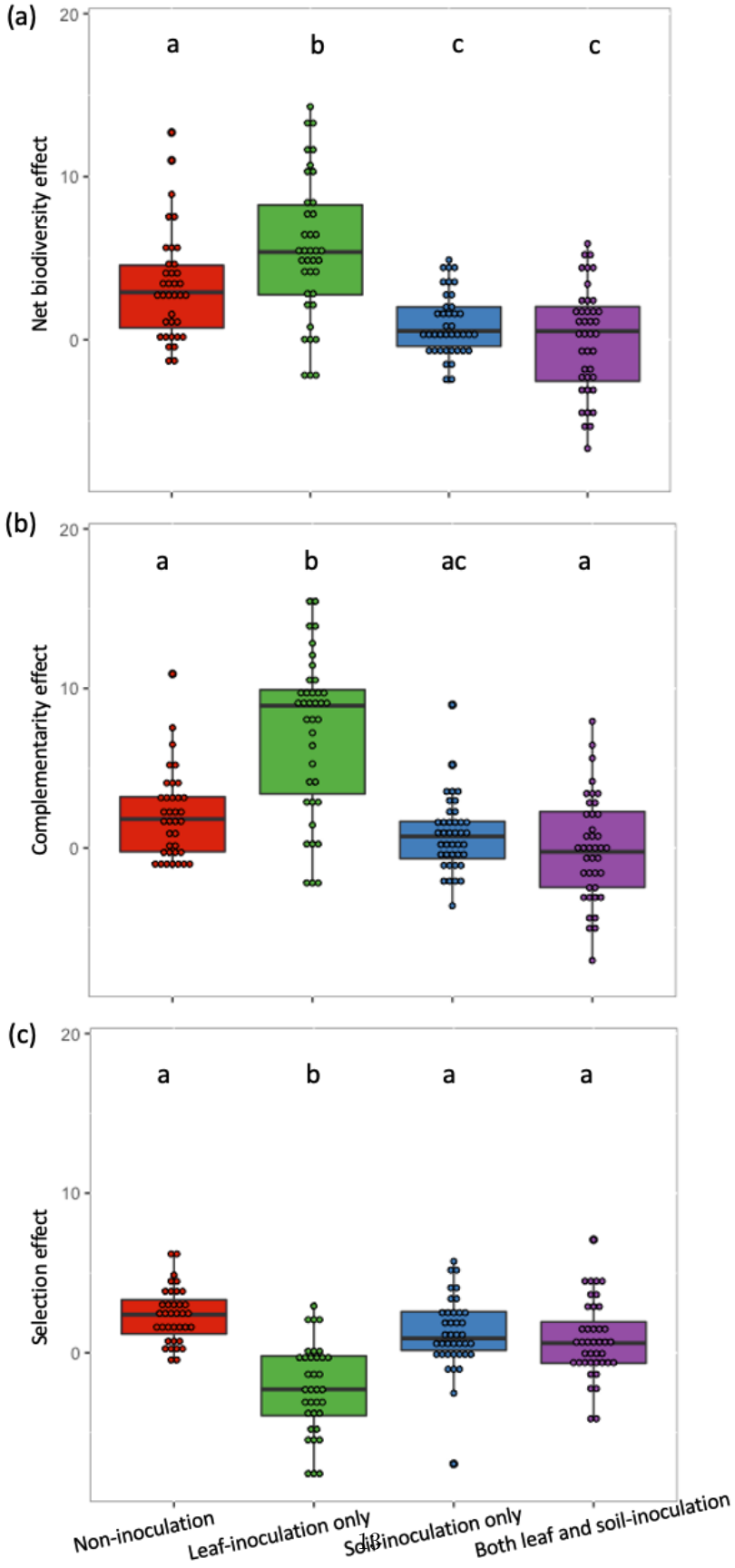

Fig. 4

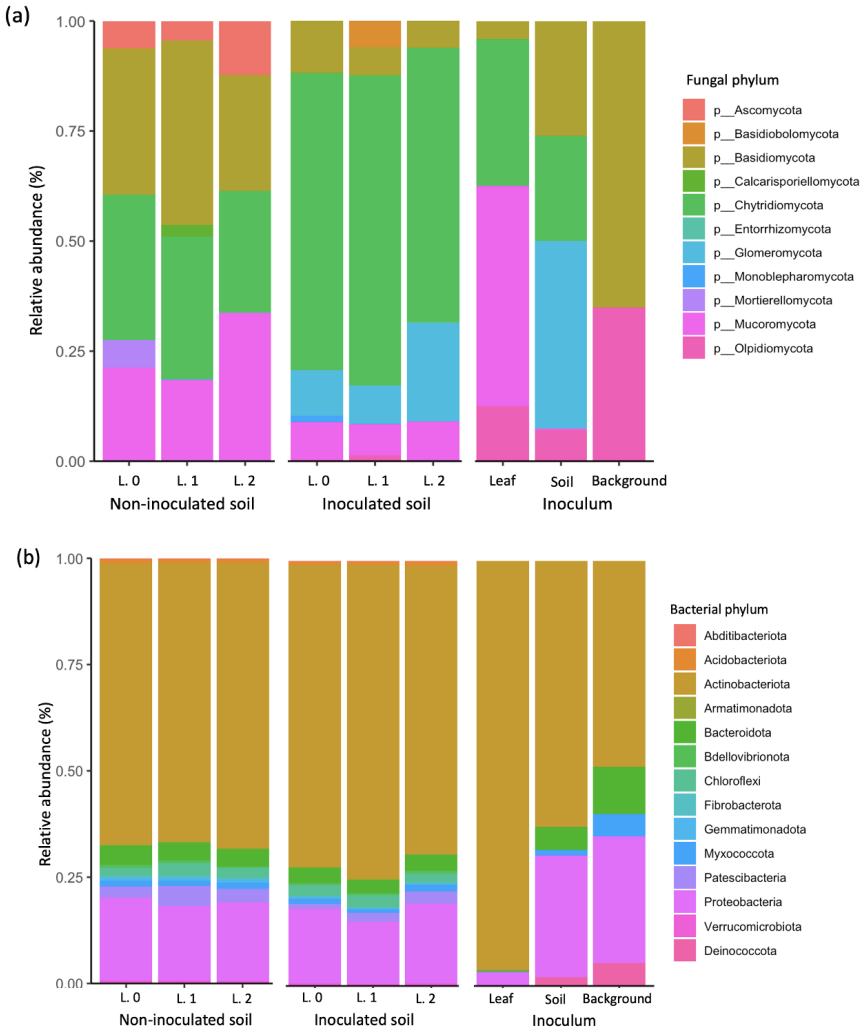

Fig. 5