Phyllosphere fungal diversity generates pervasive nonadditive effects on plant performance

Brianna K. Almeida, Elan H. Tran and Michelle E. Afkhami

Department of Biology, University of Miami, Coral Gables, FL 33146, USA

Author for correspondence:
Michelle E. Afkhami
Email: michelle.afkhami@miami.edu

Received: *28 November 2023*
Accepted: *1 April 2024*

New Phytologist (2024)
doi: 10.1111/nph.19792

Key words: antagonism, foliar microbiome, microbial interactions, nonadditive, synergism, synthetic consortia.

Summary

- Plants naturally harbor diverse microbiomes that can dramatically impact their health and productivity. However, it remains unclear how fungal microbiome diversity, especially in the phyllosphere, impacts intermicrobial interactions and consequent nonadditive effects on plant productivity.
- Combining manipulative experiments, field collections, culturing, microbiome sequencing, and synthetic consortia, we experimentally tested for the first time how foliar fungal community diversity impacts plant productivity. We inoculated morning glories (*Ipomoea hederifolia* L.) with 32 phyllosphere consortia of either low or high diversity or with single fungal taxa, and measured effects on plant productivity and allocation.
- We found the following: (1) nonadditive effects were pervasive with 56% of fungal consortia interacting synergistically or antagonistically to impact plant productivity, including some consortia capable of generating acute synergism (e.g. > 1000% increase in productivity above the additive expectation), (2) interactions among 'commensal' fungi were responsible for this nonadditivity in diverse consortia, (3) synergistic interactions were approximately four times stronger than antagonistic effects, (4) fungal diversity affected the magnitude but not frequency or direction of nonadditivity, and (5) diversity affected plant performance nonlinearly with the highest performance in low-diversity treatments.
- These findings highlight the importance of interpreting plant–microbiome interactions under a framework that incorporates intermicrobial interactions and nonadditive outcomes to understand natural complexity.

Introduction

Plants naturally harbor complex and diverse microbiomes containing many microbial taxa interacting with both their host plant and one another (Lundberg *et al.*, 2012; Roman-Reyna *et al.*, 2019; Trivedi *et al.*, 2020). Plant–microbiome interactions shape a wide range of ecological processes (e.g. succession, community assembly, and speciation; Afkhami & Strauss, 2016, Osborne *et al.*, 2017, Howard *et al.*, 2020) and contribute to many ecosystem services (e.g. carbon sequestration, nutrient cycling, and primary productivity; Averill *et al.*, 2014; Gougoulias *et al.*, 2014; Li *et al.*, 2019; Harman *et al.*, 2021). Much of our understanding of these interactions comes from decades of research and numerous manipulative experiments inoculating plants with individual microbial species of interest. These studies have repeatedly documented the importance of pathogens on plant health as well as how particular symbiotic microbes, such as *Epichloë* endophytes, rhizobia, and mycorrhizal fungi, can benefit plant productivity and fitness (Schardl, 1996; Hoeksema *et al.*, 2010; Thamer *et al.*, 2011) with cascading effects on the community dynamics of herbivores and other organisms (Hartley & Gange, 2009; Arnold *et al.*, 2014). For instance, systemic endophytic fungi living inside plants can improve host performance by priming plants against pathogens and reducing abiotic stresses, such as drought and nutrient limitation (Hubbard *et al.*, 2014; Khare *et al.*, 2018; Liu & Brettell, 2019). While manipulative studies of single microbial symbionts have provided important insights into some of the microbial effects on plants, this approach typically ignores microbial diversity and thus does not consider the importance of intermicrobial interactions that undoubtedly are happening within the microbial communities for plant performance.

Importantly, previous observational studies suggest that microbial diversity can increase plant productivity, having described positive relationships between microbial diversity and plant productivity in a variety of ecosystems. For instance, belowground research of grasslands has shown that as the diversity of arbuscular mycorrhizal fungi increases, so does plant productivity (Van Der Heijden *et al.*, 1998) and that community-wide fungal richness is positively correlated with plant richness (Yang *et al.*, 2017). Similarly, canopy density in temperate forests was found to be positively associated with ectomycorrhizal fungi richness (Lang *et al.*, 2023). However, it is challenging to experimentally test how microbial diversity and intermicrobial interactions within complex microbiomes impact plant performance due to the inherent difficulty of manipulating multiple members of these diverse communities. Some experimental studies have embraced the natural complexity harbored within plant microbiomes by manipulating the presence of whole microbial communities that vary in their community properties or experiences (e.g. using 'live' soils that vary in their richness or environmental stress legacy as microbiome inoculum; David *et al*., 2020; Korenblum *et al*., 2020; Morella *et al*., 2020; Kiesewetter & Afkhami, 2021). However, this method cannot easily determine which microbes and intermicrobial interactions underpin the observed changes in their host plants since the entire microbiome is changing at once. Recent synthesis has highlighted the importance of manipulation of tripartite interactions as a bridge between single partner interactions and whole microbiome sequencing/inoculations as well as an avenue for studying the impact of microbial interactions on plant performance (Afkhami *et al*., 2020). Specifically, these tripartite studies have been leveraged to provide preliminary insights into how nonadditivity can result from interactive effects of microbes within the microbiome. For instance, complementarity of the rewards or services that microbes provide to their host may lead to increased plant growth greater than the additive expectation (i.e. expected growth based on the benefits provided by each microbe alone; Afkhami *et al*., 2014; Hori *et al*., 2021). For example, one study revealed a higher frequency of synergistic effects on *Panicum virgatum* (switchgrass) when inoculated with pairs of fungi that differed in functional traits that were likely to provide distinct and complementary rewards (Connor *et al*., 2017). While nonadditive outcomes appear to be common based on tripartite studies (Larimer *et al*., 2010, 2014; Ren *et al*., 2016), plant microbiomes are complex with many interactions between diverse taxa. Little is known about the frequency and importance of nonadditivity for plant performance outcomes with more complex microbial communities and how the diversity of the microbiome may impact nonadditive responses to microbial interactions. One pathway forward would be experiments manipulating diversity within synthetic microbial consortia, as synthetic consortia have served to experimentally link reductionist and community-level approaches to understanding microbial communities in the past (e.g. Niu *et al*., 2017 in which synthetic consortia were used to demonstrate the impact of particular microbial taxa on bacterial community assembly in maize roots). While previous studies have often demonstrated correlative effects of microbial diversity, here, we embrace and manipulate phyllosphere fungal community complexity using synthetic consortia to determine how microbial diversity impacts nonadditive outcomes of plant performance.

Diversity within microbial communities may affect the importance of intermicrobial interactions leading to nonadditive effects on host plant productivity. In isolation, microbes may act as either pathogens, mutualists, or commensals (harming, benefiting, or having no effect on plant health, respectively) toward their hosts, but their assumed role may change depending on the context in which the interaction takes place, such as in the context of high- or low-diversity microbial communities. The net effect of all cooperative and competitive interactions between microbes can lead to three possible outcomes for the plant (Afkhami *et al*., 2020). First, the host plant may perform better than the additive expectation from singly inoculated plants due to the synergism of beneficial microbial effects. This outcome could result from complementary benefits provided by different microbes or cooperation among microbes. Second, the host plant may perform worse than the additive expectation from singly inoculated plants. This antagonism could result if members of the microbiome are in competition or conflict with one another. Third, the plant's performance may match the additive expectation of singly inoculated plants, which could occur if microbes had no measurable impact on one another and the benefits or costs to the host. This outcome would also occur if synergistic and antagonistic effects among different groups of microbes within high-diversity communities balance each other out or if the neutral effects of many microbes in high-diversity communities outweigh weak synergistic/antagonistic interactions.

In this study, we combined manipulative experiments, field collections, fungal culturing, microbiome sequencing, and synthetic microbial consortia to explore the importance of nonadditivity and diversity in microbial effects on plant productivity. We inoculated plants with 16 high- and 16 low-diversity experimental consortia of foliar fungi and compared plant performance outcomes to additive expectations (based on single microbe inoculations) to determine whether changes in the phyllosphere fungal microbiome's diversity lead to synergistic, antagonistic, or additive outcomes on plant performance. Specifically, we tested: (1) how frequently nonadditivity occurs in microbial consortia, (2) the consequences of microbial diversity for the strength and direction of nonadditive effects, and (3) the impacts of foliar fungal diversity on host productivity. Our results highlight the pervasiveness of nonadditive microbial effects on plant productivity and show that foliar fungal diversity influences the magnitude but not frequency of nonadditivity, demonstrating that interactions within the host-associated fungal microbiome can shape the interaction between plants and their microbiomes.

Materials and Methods

Study system and field collections

To determine how foliar fungal diversity impacts host productivity, we manipulated the leaf fungal community of *Ipomoea hederifolia* L., a morning glory native to the Southeastern United States. *Ipomoea hederifola* was chosen largely because access to multiple populations across South Florida allowed us to collect and evaluate the natural microbiome of this host plant and generate the culture collection from natural populations required for manipulating the consortia diversity in our experiment. It was also selected for its tractability (e.g. germinated readily) and our ability to provide appropriate growth conditions (e.g. natural light conditions and appropriate field-collected soil). *Ipomoea hederifolia* is readily found in the understory of upland habitat on the edges of the imperiled Pine Rocklands ecosystems. We collected leaves of *I. hederifolia* from 10 natural habitat patches (16 ± 2.8 plants per site; total plants sampled = 199) across

Miami-Dade County to generate fungal isolates that form natural plant–fungi associations. Collected leaves were the second fully expanded, mature leaf from the apical leaf and were from high-light environments typical of the open canopy environment of the Pine Rocklands ecosystem. Seeds for our experiment were collected from the Taylor R. Alexander Representative Species Assemblage of Natural Upland Communities of South Florida on the University of Miami campus (Coral Gables, FL, USA).

Generating culture collection of foliar diversity

Following methods from Cook *et al.* (2013), a fungal culture library was created by placing the adaxial side of two field-collected leaf fragments (*c.* 15 cm^2) face down on potato dextrose agar plate, isolating individual emergent fungal isolates, and then propagating each isolate clonally. Antibiotics (ampicillin, streptomycin, or kanamycin) were used to prevent bacterial growth and to increase the diversity of fungal taxa acquired from collected leaves, as prevention of bacterial growth improves the ability to morphologically distinguish between different fungal taxa to be sent for sequencing (Black, 2020). Each fungal isolate was identified by extracting the fungal DNA and amplifying the ITS1-LR3 region using standard Extract-N-Amp protocols (David *et al.*, 2016; Sigma Aldrich Corp., St Louis, MO, USA). Samples were then sent to Eurofins (Louisville, KY, USA) for Sanger sequencing, and the BLASTN algorithm was used to query NCBI databases for these sequences to identify the fungal isolates. To select which of these isolated and identified fungi would be used in the diversity treatments, we then sequenced the whole foliar fungal microbiomes from the field-collected *I. hederifolia* leaves and selected the 20 unique fungal taxa from the culture collection that were most relatively abundant among the overall fungal communities (an indicator of dominance).

To profile the natural foliar fungal microbiome, we performed DNA extractions from a subset of our field-collected leaves that were representative of the range of our collections (28 extractions, 2 leaves/extraction; Qiagen DNeasy Plant mini kit Cat No.: 69204) and used two-step dual indexing to prepare barcoded amplicon libraries of the ITS region (using ITS1F and ITS4 primers; White *et al.*, 1990; Gardes & Bruns, 1993) as well as a negative control library (using Ultrapure water in lieu of leaf tissue during extraction; Gohl *et al.*, 2016; Revillini *et al.*, 2022). Libraries were sequenced on the ILLUMINA MISEQ PLATFORM (v.3, paired-end 300-bp) at the University of Miami Center for Genome Technology. Sequences were demultiplexed (*bcl2fastq*), denoised, and grouped into OTUs (operational taxonomic units) based on 99% similarity using the QIIME2 (v.3) pipeline and then rarified fungal sequences to 1000 reads at which rarefaction curves reached saturation. Taxa were identified with the UNITE database (v.01-12-2017; UNITE Community, 2017), and the cultured fungal taxa were compared with fungi in the resulting community-wide taxa matrix to select which isolated fungi would be used in the experiment based on their prevalence in multiple samples and abundance in the microbiome.

Experimental setup and data collection

To test the effects of fungal consortia diversity on plant productivity, we surface sterilized *I. hederifolia* seeds with 0.15% tebucanozole solution (as in Kucht *et al.*, 2004) and then germinated the seeds in sterile Petri plates with moist filter paper. Eight days after germination, seeds were aseptically planted in pots (262 ml Heavyweight Deepots; Stuewe and Sons, Corvallis, OR, USA) of sterile soil (autoclaved three times at 121°C for 2 h with 24 h between each autoclave cycle; Huangfu *et al.*, 2022) in the glasshouse at the University of Miami campus. Three weeks postgermination, leaves were abraded with sterilized sand to create openings in the leaf surface and sterile cotton gauze was used to inoculate plants (Subedi *et al.*, 2022) with either a single fungus from our culture collection (i.e. monocultures), a 'low' microbial diversity treatment (i.e. simple synthetic consortia of 3 fungal taxa), a 'high' diversity treatment (i.e. relatively more complex synthetic consortia of 10 fungal taxa), or a sham inoculum of sterile water (i.e. control treatment with no microbial addition). We used a replacement design experiment based on best practices from previous diversity–productivity studies (Tilman *et al.*, 1996; Symstad *et al.*, 1998; Huston & McBride, 2002; Crawford & Rudgers, 2012). We utilized a replacement design (over an additive design) in order to hold total fungal abundance constant and only manipulate fungal diversity (Huston & McBride, 2002; Bybee-Finley *et al.*, 2016; Li *et al.*, 2023). Previous studies comparing these designs found that using a replacement design does not limit the ability to detect nonadditive effects and the overall effects of communities on plant biomass (Špaèková & Lepš, 2001). We created inocula for each of the 20 fungal taxa from our culture collection (Table 1; Christian *et al.*, 2019), which were applied to 16 replicate monoculture plants per fungal isolate. The sterile water control (sham 'no microbe' inoculum) was also applied to 16 plants. The two diversity treatments were generated by randomly selecting combinations of fungi from the pool of 20 isolated fungal taxa, resulting in 16 distinct low-diversity (three taxa in each) experimental consortia and 16 distinct high-diversity consortia (10 taxa in each). Our pool of 20 fungal cultures was sufficiently large to generate unique consortia compositions in both the low- and high-diversity treatments, which is important for preventing pseudoreplication by testing the effects of fungal diversity rather than taxonomic composition (Huston, 1997; Hicks *et al.*, 2011). Each of these 32 synthetic microbial consortia (16 high- and 16 low-diversity consortia) was inoculated into three plants.

To create the inocula, we suspended hyphal fragments from each fungal culture plate in 50 ml of sterile water by vortexing for 3 min, and then, we determined the concentration of hyphal fragments using a cytometer and diluted all fungal isolate solutions to equal concentrations by adding sterile water (1.37×10^5 hyphal fragments ml^{-1}). These solutions were then used for monoculture inocula and to generate 15 ml of inoculum stock solutions of high- and low-diversity community treatments by combining the appropriate single isolate inocula in equal volumes. For example, a low-diversity inoculum combined 5 ml of each of three randomly chosen monoculture inocula and a

Table 1 Information for the 20 fungi that were cultured, identified, and used in our synthetic consortia.

Isolate ID	Blast % identity	Accession	Taxa ID
1	99.79	MG838060.1	*Fusarium 1* (*F. pseudocircinatum*)
2	100.00	MK280817.1	*Fusarium 2* (*F. citri*)
3	98.26	HM060271.1	*Phialemonium* (*P. dimorphosporum*)
4	96.53	JX402138.1	*Collectotrichum* (*C. gloeosporioides*)
5	97.78	MN644790.1	*Trichoderma 1* (*T. pubescens*)
6	99.68	MG274295.1	*Fusarium 3* (*F. proliferatum*)
7	99.69	MT453296.1	*Fusarium 4* (*F. oxysporum*)
8	99.74	LN898719.1	*Aspergillus* (*A. sydowii*)
9	99.74	MT487842.1	*Talaromyces* (*T. aurantiacus*)
10	99.90	MT476857.1	*Curvularia* (*C. senegalensis*)
11	99.00	KF436183.1	Fungal endophyte culture collection STRI:ICBG-Panama
12	100.00	KF673689.1	Fungal endophyte voucher Arizona
13	100.00	MN452167.1	*Fusarium 5* (*F. chlamydosporum*)
14	100.00	MF379655.1	*Talaromyces* (*T. verruculosus*)
15	99.79	GU219467.1	*Sarocladium* (*S. strictum*)
16	99.17	MN080416.1	*Fusarium 6* (*F. solani*)
17	99.08	MN540202.1	*Trichoderma 2* (*T. atroviride*)
18	99.13	MN626679.1	*Fusarium 7* (*Fusarium* sp.)
19	99.79	KF746131.1	*Fusarium 8* (*Fusarium* sp.)
20	99.90	GQ505438.1	*Fusarium 9* (*Fusarium* sp. NRRL)

The 'Taxa ID' column lists the genus identification of each culture as the primary level of classification followed by the closest NCBI species-level match in parentheses, which was determined using the highest % identity match to the ITS1-LR3 regions obtained from Sanger sequencing of culture fungi (mean *e*-value of matches = 7.85 × 10–34). The 'Accession' column provides the related NCBI accession numbers for the closest NCBI matches and the 'Blast % identity' column quantifies overlap in the cultures and accessions' sequences. Note that species-level classifications are putative since one locus was used in identifying the closest NCBI match.

high-diversity inoculum combined 1.5 ml of each of 10 randomly chosen inocula. Sterile gauze was used to apply these inocula to each plant (*c.* 2 ml total volume of inoculant per plant). For more information on the composition of these communities, see Supporting Information Tables S1 and S2. We also treated 16 plants with a leaf slurry inoculum made by aseptically homogenizing field-collected leaves containing the natural fungal microbiome of *I. hederifolia* following Subedi *et al.* (2022) (homogenizing field-collected leaves in sterile water using a bleach-sterilized blender) to ensure that the effects of synthetic fungal consortia in our experiment were comparable to the microbial effects experienced by plants with natural foliar fungal microbiome communities.

The experiment was harvested 5 months after germination. Plant height (i.e. length of the vine) as well as above and belowground biomass were measured. After root tissue was washed to remove soil, above and belowground biomass were dried separately at 60°C to a constant weight and then measured (Mettler Toledo ME-T Analytical Balance, Columbus, OH, USA). Total biomass was determined by taking the sum of above and belowground biomass. Sequence data and plant performance data have been submitted to NCBI (PRJNA874722) and Zenodo (doi: 10.5281/zenodo.7032799).

Statistical analyses

Before performing statistical analyses, we assessed normality using Shapiro–Wilk tests and determined that log transformations were needed to improve normality for all performance data. To investigate whether individual fungal taxa are mutualistic, commensal, or pathogenic, we compared the performance of monoculture-inoculated plants to that of noninoculated control plants with a MANOVA and follow-up ANOVAs of individual performance metrics. For classifying taxa's interactions with plants, we use the results from these MANOVAs (95% CIs also present in the graphical representation of the results).

To determine whether plant response to endophyte diversity is nonadditive, we performed Monte Carlo simulations. The Monte Carlo simulation generated a distribution of 9999 possible expected additive outcomes by repeatedly randomly sampling growth values from the single inocula treatment plants that correspond to the fungal isolate combinations in that consortia and averaging these values to calculate the consortia mean effect on plant growth. We then asked whether the growth value for plants in our experiment with that synthetic consortia was significantly different from the additive expectation by comparing the actual value to the 95% confidence intervals determined from the Monte Carlo generated distribution. If the actual mean plant performance from a high- or low-diversity treatment community fell outside of these intervals, then the effect of fungal consortia combination on plant performance was determined to be nonadditive (Crawford & Whitney, 2010). We quantified how many times a foliar fungal consortium contributed to an antagonistic (≤ bottom 2.5% of the distribution) or synergistic effect (≥ top 2.5% of the distribution) on any plant performance metric. We then investigated how likely it was for low- or high-diversity treatments to lead to antagonisms or synergisms on multiple plant traits using a chi-square analysis with the frequency of antagonisms or synergisms as the response and diversity treatment as the predictor. To determine whether the magnitude of positive or negative effects on plant performance varied between low- and high-diversity treatments, we first standardized each performance metric (standardized normal deviates) and calculated expected performance of plants grown with diversity treatments using the average performance values from plants grown with monoculture treatments. We performed an ANOVA with diversity treatment (i.e. low- vs high-diversity synthetic consortia) and direction of positive or negative effects compared with expected, as well as their interaction and consortia identity as categorical predictors and magnitude of effect (i.e. deviation in plant performance with diverse synthetic consortia from expected values based on additive effects from the monocultures) as the response variable.

To understand the relationship between foliar fungal diversity and plant growth, we analyzed the effects of fungal diversity on multivariate plant performance by performing a MANCOVA with follow-up analyses on each performance metric using the same explanatory variables (after confirming significant overall microbial effects). The model included diversity treatments level as a continuous metric and synthetic consortia identity (nested within diversity treatments) as explanatory variables and plant growth metrics of root mass, shoot mass, and height as the response variables. We also conducted ANOVAs on plant investment (root-to-shoot ratio) or total biomass as response variables with diversity level and synthetic consortia identity nested within diversity level. Finally, we performed trend contrast analysis to evaluate the shape of the relationship between fungal diversity and plant growth. All statistical analyses were performed in R v.3.6.0. (R Core Team, 2021).

Results

Natural phyllosphere microbiome

Across the 28 fungal communities sampled from field-collected host plant leaves, we found a total of 1170 fungal exact sequence variants (ESVs) and 324 putative fungal species (i.e. grouped at the 99% sequence similarity). The mean ESV richness (i.e. number of ESVs per sample) was 110 ± 10 ESVs per plant sampled (mean ± SE) and the mean ESV Shannon diversity was 2.9 ± 0.14. When we grouped reads by 99% sequence similarity, richness at each site was 62 ± 1 and diversity was 2.5 ± 0.13. Fungi from the genus *Cladosporium* were the most common across all samples, present in 27 out of 28 host plants with a mean relative abundance of *c*. 7%. Taxa from the genera *Phoma* and *Hygrocybe*, which are often pathogens or saprotrophs, were also among the most commonly detected fungi.

We further investigated the natural fungal community using FUNGuild to assign genera to functional guilds. The community was separated into nine total guilds (Fig. S1). The most abundant guild was the 'Animal Pathogen-Endophyte-Epiphyte-Plant Pathogen-Undefined Saprotroph' guild. Due to the nature of this assignment, the fungi in this category could function as any of these guild assignments, making it difficult to discern their possible impacts on their plant host. However, the second most abundant guild, comprising 20% of the taxa guild identifications, was 'Plant Pathogen'. Many of the taxa in the culture collection (Table 1) are also often characterized as pathogenic fungi in the literature, showing consistency between our culture collection and the natural community guild affiliations. However, we did not detect any disease symptoms of host plants during leaf sample collection in the field nor in our inoculated plants during our experiment. The cultures used in our synthetic consortia experiment matched microbes from our host community-wide data set with a sequence overlap of 98% ± 0.4 and an *e*-value of $7.85 \times 10^{-34} \pm 4 \times 10^{-34}$ (Fig. S2).

Fungal taxa functioned as commensals in isolation

We taxonomically identified the 20 foliar fungal isolates used in our experiment (Table 1), which included taxa that have been characterized in other systems as plant pathogens (e.g. *Fusarium oxysporum* and *Colletotrichum gloeosporioides*) and saprotrophs (e.g. *Aspergillus sydowii*) as well as fungi characterized as having multiple lifestyles (e.g. *Sarocladium strictum* which has been characterized as both a pathogen and saprotroph). However, because microbial roles often differ among host plant species (Long *et al*., 2008; Šmilauer *et al*., 2020) and because most of the identifications were based on studies of crops (which can be under significantly different ecological and evolutionary pressures; Kiers *et al*., 2007; Malcolm *et al*., 2013; Millar *et al*., 2023), we experimentally characterized the effects of each fungus on our host plant. When we compared the effect of single taxa inoculations to the control treatment, we found that all 20 fungi acted as commensal symbionts when inoculated alone (Fig. 1), as they did not significantly change plant performance compared with the uninoculated control plants (e.g. Total Biomass $F_{20,139} = 0.776$, $P = 0.739$; see Table S3 for more details). Interestingly, the average effects on plant performance of many of the cultures were below the '0' line in Fig. 1(a,b) and above the '0' line in Fig. 1(c), suggesting a nonsignificant trend of weak negative effects on root and shoot mass and weak positive effects on plant height of single strain inoculations. While we consider these microbes to be effectively 'commensals' throughout the paper (based on the statistical results from the MANOVA with single culture inoculations which corrects for multiple comparisons), we are also careful to use the actual measured effect of each microbe on plant performance in subsequent analyses testing for nonadditivity (which accounts for any possible weak negative or positive effects among monocultures when determining whether synergism or antagonism occurred).

Foliar fungal diversity impacts plant performance

Interestingly, the effect of fungal diversity on total plant biomass (but not its component variables) followed a quadratic relationship, (trend contrast: $t_{204} = -2.11$, $P = 0.036$; Fig. 2). Plants inoculated with a low diversity of fungal isolates had the greatest total biomass followed by plants inoculated with a high diversity of fungal isolates and singly inoculated plants having the next

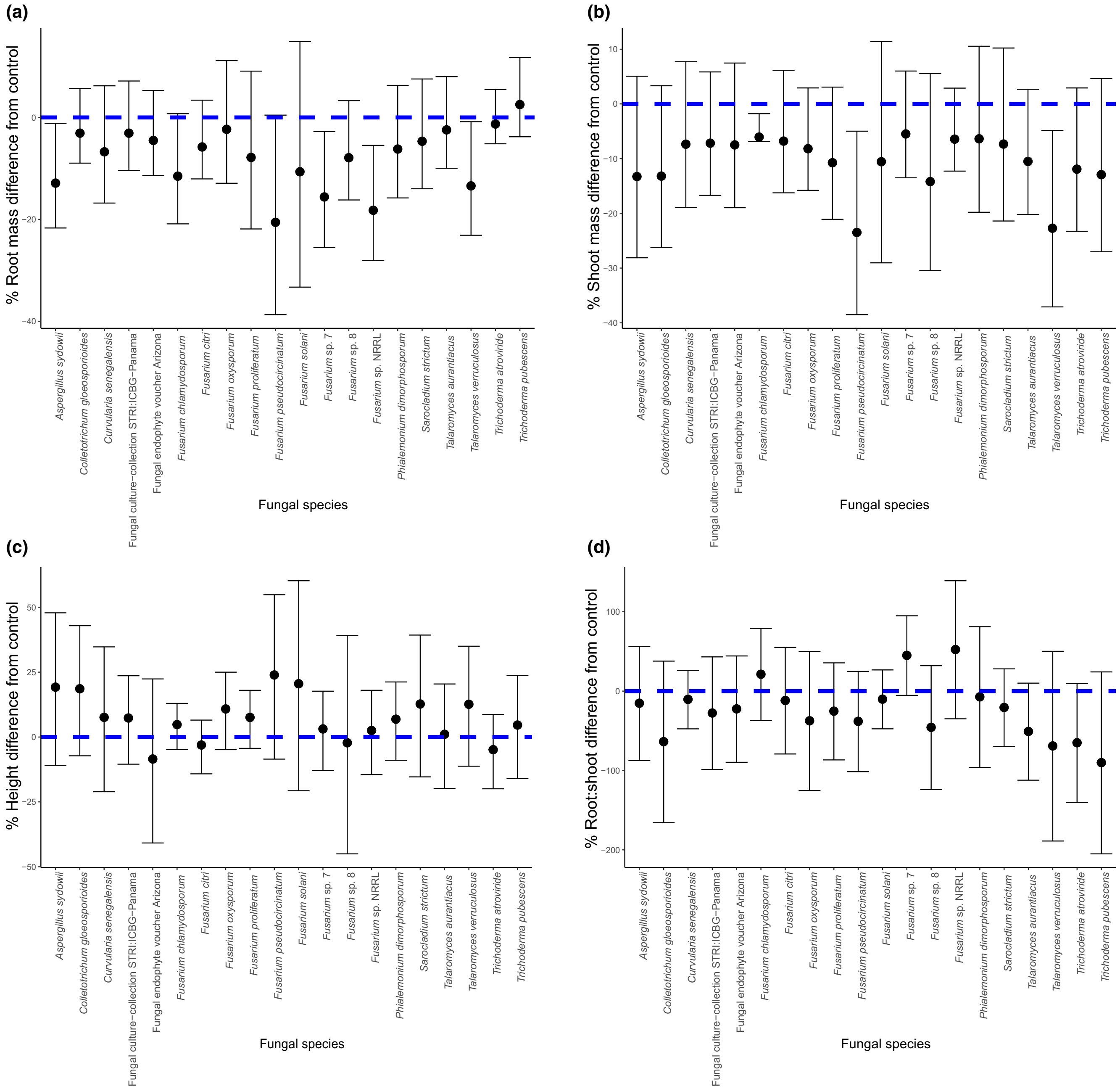

Fig. 1 All fungal taxa used in the experiment acted as commensals when singly inoculated. This figure depicts the percent difference in the growth effects of each fungus compared with the control treatment (plants treated with sterile 'sham' inoculant) for (a) root biomass (b) shoot biomass, (c) height, and (d) investment in roots vs shoots (i.e. root-to-shoot ratio). Each point represents the mean percent difference for plants grown with one of the 20 fungal taxa, and error bars represent 95% CIs around the mean. The mean percent difference was calculated by taking the mean of the difference in each outcome for the monoculture treatment and the mean of the control, then dividing by the mean of the control. Note that while the 95% CIs show a few taxa's effects bounded away from 0 for biomass effects, the statistical results from the ANOVA test (available in Supporting Information Table S5) found that none of the fungi's effects on plant performance were significantly different from the control plants. We use the results from the ANOVA over 95% CIs and because the ANOVA accounts for experiment-wide error rates (Crawford & Whitney, 2010).

highest, and by sham-inoculated, control plants having the lowest total biomass. Additionally, the identity of the foliar fungal consortia significantly affected overall plant performance ($F_{48,158} = 1.28$, $P = 0.029$; MANCOVA results including root mass, shoot mass, and height see Fig. S3), and total biomass produced ($F_{49,158} = 1.67$, $P = 0.009$). In addition, all performance metrics for high- and low-diversity consortia were significantly different from control (Tables S4, S5; Fig. S4). This demonstrates that plants can benefit from even very low-diversity phyllosphere communities compared with single species inocula,

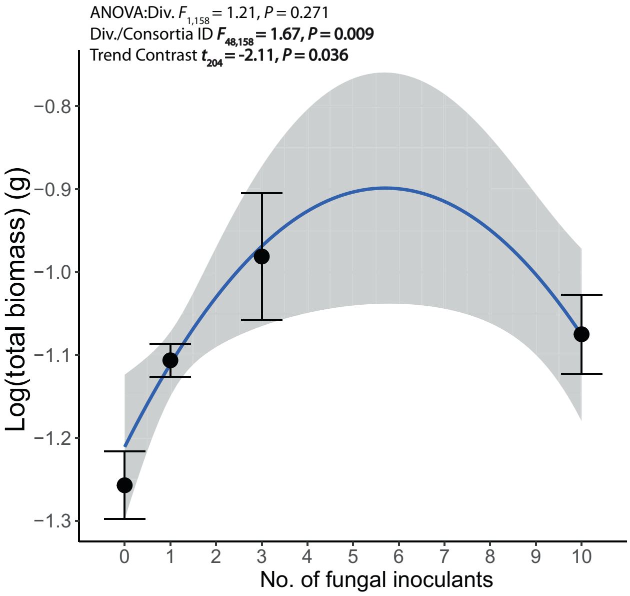

Fig. 2 Host plant biomass follows a quadratic relationship with an increased number of taxa in the fungal consortia treatments (trend contrast: $t_{204} = -2.11$, $P = 0.036$). Points with bars indicate mean plant performance (i.e. log(total biomass) ± SE). The gray shading indicates the 95% confidence interval of the blue trendline.

showing promise for the use of synthetic consortia to promote plant growth even with the limitations of creating these communities.

Fungal communities composed of commensals have nonadditive effects on plant performance

Plants inoculated with experimental fungal consortia performed similarly to plants inoculated with the whole natural microbiome via the slurry method, with 91% of the root mass and 81% shoot mass outcomes from plants grown with synthetic fungal consortia falling within one SD of performance of plants grown with leaf slurry (whole microbiome-treatment) plants (Fig. S5). This suggests that the synthetic consortia can provide a realistic model for microbial effects on plant performance.

We then used Monte Carlo analysis comparing the performance of plants grown with synthetic fungal consortia to the distribution of plant performances predicted based on single inocula treatments, finding that nonadditive effects of fungal symbionts were ubiquitous. In fact, more than half of the synthetic consortia (56% of 32 consortia) had significant nonadditive effects on at least one host plant performance metric and all four plant traits responded nonadditively to at least one synthetic consortia (Figs 3, S6). Furthermore, both synergistic and antagonistic effects on plant performance (i.e. positive and negative nonadditivity, respectively) were common, with 10 consortia having synergistic effects and 9 having antagonistic effects at least once. We also noted several instances of 'acute synergism', which are cases with extreme and unexpectedly high levels of synergism measured as nonadditive instances > 300%. Acute synergisms in this study included one case where plant productivity across all three growth metrics was over 1000% greater than the additive expectation based on the fungal effects on plant growth when in monoculture inoculations (Fig. 3; consortium 3) and another fungal consortium that increased productivity by 350–600% across all productivity metrics (Fig. 3; consortium 13).

We measured the frequency of nonadditive outcomes across our four performance metrics in Fig. 3. There was an equal number of high- and low-diversity consortia causing nonadditive outcomes on at least one plant performance metric (9 of 16 low-diversity communities and 9 of 16 high-diversity communities); therefore, the probability of nonadditive effects on plant growth did not depend on the diversity of the microbial consortia (Fig. 3). Furthermore, when dividing nonadditivity into synergistic and antagonistic effects on plant performance, we found that low- and high-diversity fungal communities have an equal chance of nonadditively harming/benefiting plants ($\chi^2 = 0.29$, $P = 0.867$, df = 2). Specifically, there were nine instances of significant synergistic effects on plant performance generated by five different *high*-diversity fungal consortia and 11 instances generated by six different *low*-diversity fungal consortia. Similarly, we found significant antagonistic effects in 11 instances across seven high-diversity fungal consortia and six instances with five low-fungal-diversity consortia (Fig. 3). Interestingly, negative nonadditive effects were absent for shoot mass in both low- and high-diversity treatment plants, but we did find seven instances of significant synergistic effects on shoot mass. We also note that while there was an equal frequency of synergistic and antagonistic nonadditive effects generated by our synthetic consortia for most traits, synergistic effects were 4.24 times stronger on average than antagonistic effects (Figs 3, S6), suggesting that beneficial intermicrobial interactions play an important role in fungal microbiome–plant interactions.

We then investigated whether there was a difference in the strength of effects on plants of high- and low-diversity treatments using the deviation between the observed effects of synthetic fungal consortia on plant performance and the expected performance (based on the average of the monoculture treatments). We found that the magnitude of positive and negative effects on host plants was significantly different when grown with high- vs low-diversity foliar fungal consortia ($F_{1,85} = 11.48$, $P = 0.0012$; Fig. 4). Low fungal diversity treatments had a greater deviance from expected performance in both positive and negative directions, meaning that low-diversity synthetic consortia had stronger effects on plant growth compared with the higher diversity fungal consortia. We also found that variance in this deviation from expected performance was greater for plants in the low-diversity treatment than in the high-diversity treatment ($F_{1,33} = 4.87$, $P = 0.029$). Taken together this suggests that low-diversity microbial consortia may contain groups of interacting microbes that have substantive effects on plant performance, which are possibly diluted in the higher diversity treatment. This dilution may result from the balancing of synergistic and antagonistic effects of different groups of microbes within the more diverse consortia or neutral effects of many microbes outweighing any synergistic/antagonistic interactions.

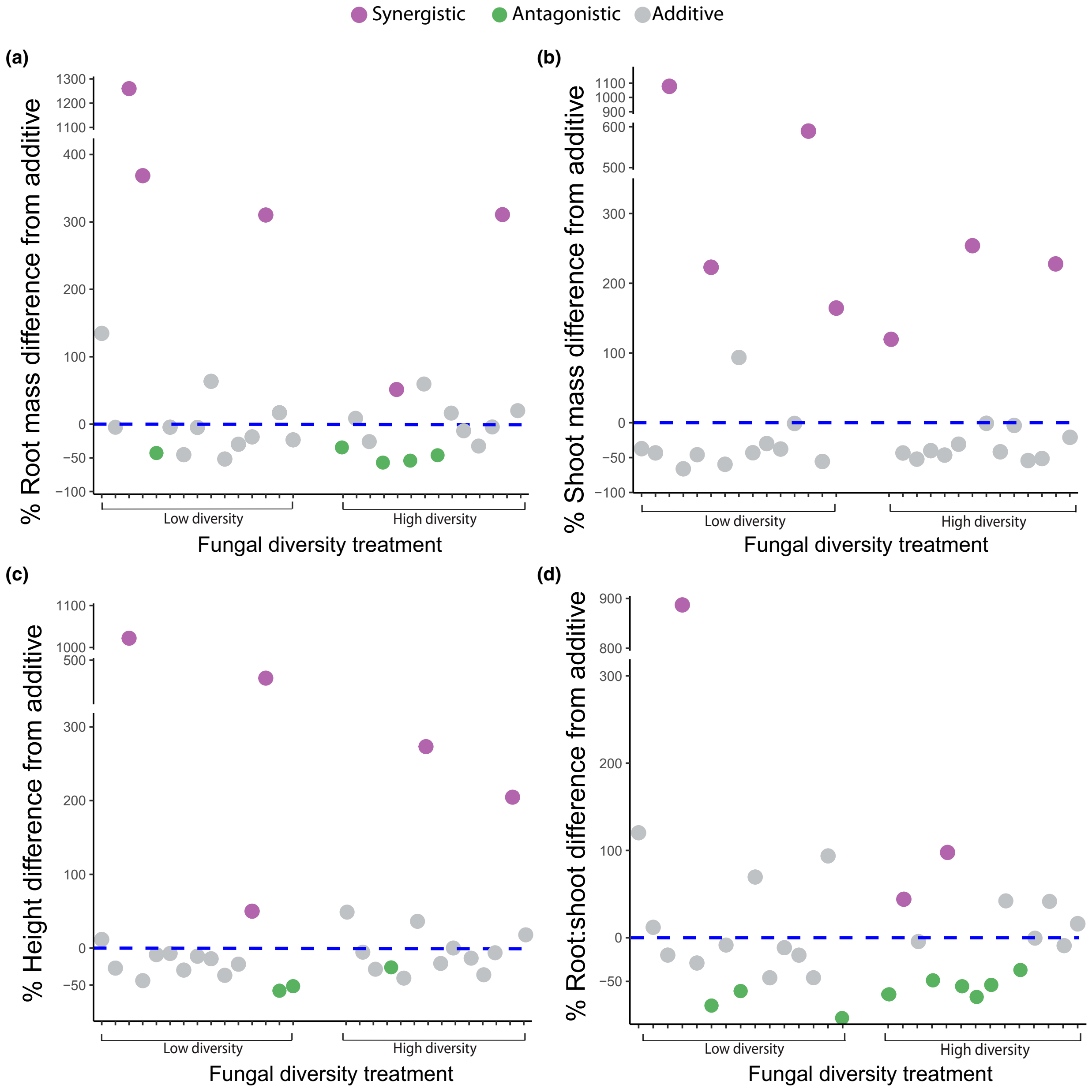

Fig. 3 Nonadditive effects of microbial consortia on plant performance metrics of (a) root biomass, (b) shoot biomass, and (c) height as well as on (d) plant allocation between roots and shoots (root-to-shoot ratio). Each point represents the difference between an experimental consortium's effect on a given plant trait and the additive expected trait value calculated based on how the taxa that make up the consortium affected plants in the monoculture treatments (i.e. when singly inoculated). Purple points indicate synergistic effects on plant productivity, green points indicate antagonistic effects, and light gray points indicate additive consortium effects. The order of the low- and high-diversity microbial consortia is consistent across panels. The mean percent difference was calculated by taking the mean of the difference between each diversity treatment outcome and the mean of the additive expectation divided by the mean of the additive expectation.

To gain additional insight into microbially mediated nonadditivity, we investigated which fungal taxa make up consortia that had nonadditive effects on host plants in our experiment. We found that, while no individual fungal taxon was particularly common in consortia that led to both types of nonadditive effects (i.e. both synergistic and antagonistic outcomes; $\chi^2 = 22.2$, $P = 0.27$, df = 19), several fungal taxa were notably frequent in synergistic consortia *or* in antagonistic consortia. For instance, an *Aspergillus* isolate (closest BLAST match to *Aspergillus sydowii*, which is commonly a saprotroph or pathogen; Geiser *et al.*, 1998; Jiménez-Gómez *et al.*, 2020) and two fungal endophytes (closest match to vouchered specimens from Panama and Arizona) were the most consistently and frequently found in synergistic consortia (Fig. S7; Table 1). In fact, these taxa were on average 41% more likely to be in consortia that had synergistic effects on plant growth than antagonistic effects (*A. sydowii*: 25%

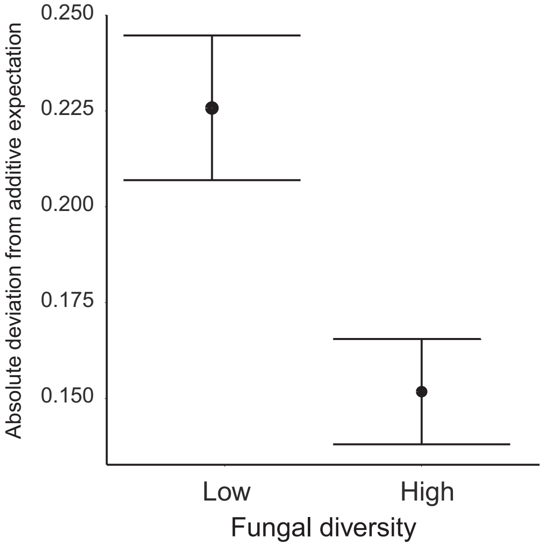

Fig. 4 Increasing foliar fungal diversity decreased the magnitude of consortia effects among microbes on plant performance. We found that the deviation between plants inoculated with experimental consortia and the additive expectation was on average greater in the lower diversity treatment (Diversity effect: $P = 0.001$). The deviation on the y-axis was determined by standardizing plant performance values (converting into standardized normal deviates to allow comparison of effect across traits that differ in scale) and calculating the difference between each experimental consortia effect on plant performance and the additive expectation based on the monoculture performance outcomes. Points with bars indicate mean ± SE.

of consortia are synergistic, fungal endophyte from Panama: 30% synergistic, and fungal endophyte from Arizona: 27.7% synergistic). Furthermore, their fungal consortia were not only consistently synergistic for aboveground plant growth, but also showed a greater frequency of synergistic (over antagonistic) effects on belowground plant growth, which is interesting since belowground synergistic outcomes were generally much rarer (compared with belowground antagonistic effects) across all other fungal taxa (Fig. S7). By contrast, two fungi – a *Fusarium* isolate (closest BLAST match to *F. chlamydosporum*, which is a known pathogen of multiple species of plants; Ivic, 2014; Omar *et al*., 2018) and a *Trichoderma* isolate (closest match to *T. pubescens*) – had the highest and most consistent occurrence in consortia with antagonistic effects on plant traits. Not only were 30.5% of the nonadditive consortia with *F. chlamydosporum* and 27.5% of the nonadditive consortia with *T. pubescens* antagonistic, but the magnitudes of their antagonistic effects were on average both 60% stronger than their synergistic effects (Fig. S8). The high representation of these taxa in nonadditive consortia make them candidate microbial 'catalysts' for nonadditivity (i.e. taxa that encourage synergistic or antagonistic intermicrobial interactions within consortia).

Discussion

To overcome the complexities of understanding microbial communities' fungal diversity effects on plant performance, we used experimental synthetic fungal consortia to determine how increasing microbial diversity impacts the nonadditive effects of microbes on plant performance. This study demonstrates five main results that contribute to our understanding of microbial diversity and nonadditivity as follows: (1) fungal isolates that act as commensals alone often have significant positive and negative effects on plant performance when in microbial consortia, (2) nonadditivity is a common property of microbial communities with equal amounts of synergism and antagonism, (3) synergistic effects were often stronger than antagonistic effects on plant productivity, (4) the strength, but not the frequency, of nonadditivity was affected by microbial consortia diversity, and (5) diversity's effects on plant biomass followed a quadratic relationship, with the lower diversity fungal consortia supporting the greatest total plant biomass.

Intermicrobial interactions among commensals can underpin pervasive nonadditivity in plant–microbiome interactions

All commensal microbes in our study participated in consortia with nonadditive effects on their host plant productivity, demonstrating interactions within fungal microbiomes have consequences for plant performance. Notably, despite all these fungal isolates being effectively commensal in pairwise interactions with the plant (i.e. in monocultures), this result suggests that the presence of other fungi causes many commensals to shift their relationship with their plant host, resulting in changes to plant performance.

Our results also highlight that nonadditivity is a pervasive feature of microbial communities as more than half of our randomly generated synthetic consortia had nonadditive effects on at least one plant performance trait. Direct or indirect intermicrobial interactions could have led to this nonadditivity through three (nonmutually exclusive) pathways. First, the presence of other community members changes the biotic context in which the interaction between a microbe and plant takes place, and this change in context can cause a microbe that is seemingly commensal in monoculture to confer benefits or act antagonistically when part of a natural microbial community. For example, a commensal microbe may have negative nontarget effects on host performance when engaging in competition with other microbes or conversely may become able to confer new or enhanced benefits to host plants when in the presence of other microbes that provide resources it needs. For instance, arbuscular mycorrhizal fungi can improve the ability of rhizobia to fix nitrogen by increasing the phosphorus uptake of the host plant (as N-fixation is a phosphorus-hungry process; Püschel *et al*., 2017). Second, the presence of other microbes in a community may alter the value of rewards or services that a microbe provides to its host plant. For example, many fluorescent foliar *Pseudomonas* spp. induce an immune response that can prevent infection by fungal pathogens (Van Wees *et al*., 2008), but the defensive effects of priming are only valuable to the host in the presence of pathogens. Third, multispecies interactions between microbes can provide novel functions for their host plant that lead to nonadditive effects on plant growth. For example, co-inoculation of *Capsicum annuum* with *Acinetobacter* sp. and the putative plant pathogen *Phytophthora capsici* increased the fresh weight of seedlings in an experiment. The researchers suggest this outcome resulted from changes in volatile organic compounds released by *Acinetobacter* in response to interactions with *P. capsici* that increased root length leading to greater fresh weight (Syed-Ab-Rahman *et al*., 2019). All three of these pathways are likely to play important roles in the nonadditivity found in our study and in many other plant–microbiome interactions. Future work pinpointing when each mechanism is most important would be valuable for improving the predictability of microbial effects on plants and for applied goals requiring the engineering of synthetic consortia in natural and human environments (De Souza *et al*., 2020; Sharma & Shukla, 2020).

Importance of microbial synergism and antagonistic effects on plant productivity

Using synthetic assemblies of microbes to understand the effects of microbial communities on plant performance (Großkopf & Soyer, 2014; Dolinšek *et al*., 2016), we show that not only is nonadditivity ubiquitous but also that synergism and antagonism both occur often. While the frequency of these nonadditive effects was the same regardless of diversity level, the strength of their effects on plant performance varied with microbial diversity level with stronger and more variable effects occurring in the low-diversity treatment. Interestingly, synergistic effects were also approximately four times greater in magnitude on average compared with antagonistic effects, and we found a (nonsignificant) trend of positive effects of interactions within microbial consortia being 10% greater than negative effects, on average, across all plant performance metrics and all synthetic consortia. In addition, the absence of negative nonadditive effects on shoot mass suggests beneficial nonadditive outcomes from microbial interactions may be especially common in the part of the plant that this microbial community inhabits. From the perspective of the host, these results indicate that not only is plant performance at least partially determined by nonadditive intermicrobial interactions, but also that host plants may experience stronger beneficial nonadditive effects from microbial communities than negative nonadditive effects, especially for aboveground growth. Importantly, our results documented multiple synthetic consortia that had especially strong synergistic effects on plant productivity. For instance, one consortium increased all three plant productivity metrics – plant height, root biomass, and shoot biomass – by > 1000% compared with the additive expectation and another microbial consortium increased productivity by 350–600% across all productivity metrics (see community 3 and community 13 in Fig. 2a), emphasizing how important synergism among microbes can be for plant productivity. The consortium with the > 1000% performance increase contained a fungal endophyte (most closely matches to a previously detected endophyte in a tropical woody plant; vouchered accession KF436183.1), a *Sarocladium* isolate (closest match to *Sarocladium strictum*), and an isolate of *Fusarium* (KF746131.1; Table 1). *Sarocladium strictum* and *Fusarium* are often saprotrophs but also can act as plant pathogens suggesting that extreme synergistic effects can arise from interactions among taxa not typically considered plant mutualists (Rivera-Varas *et al*., 2007; Okungbowa & Shittu, 2012). Given the strength of synergisms well beyond typical expectations for cooperative benefits, we hypothesize these synthetic consortia with this acute synergism are candidates for understanding novel functions driven by intermicrobial interactions. Future work functionally profiling synthetic consortia like these could be valuable when considering microbiome engineering.

When compared with previous tripartite studies (between a plant and two microbes), nonadditivity remains as important – if not more so – when plants interact with the more diverse experimental consortia used in our study. These synthetic consortia also resulted in some plant performance outcomes that differ from tripartite studies. For instance, in contrast to the equal frequencies of synergism and antagonism we found, tripartite studies have often reported greater rates of synergism compared with antagonism when plants are inoculated with pairs of microbes (Larimer *et al*., 2014; Connor *et al*., 2017; Wężowicz *et al*., 2017) or in a few cases greater instances of antagonism on plant performance (Barrett *et al*., 2015; Ballhorn *et al*., 2016). However, the pattern of particularly strong synergistic effects in our study is in line with outcomes from tripartite studies where the strength of synergistic effects compared with additive expectations has been as high as 180% and 238% (Larimer *et al*., 2014; Connor *et al*., 2017). Yet, the unprecedented strength of the acute synergism (e.g. > 1000% compared with the additive expectation) generated by a few microbial consortia in our study far surpasses these previous highs from tripartite studies, prompting the need for future work to manipulate diversity levels of synthetic consortia across different systems to determine how common these ‘acute synergism’ events are, what factors promote these outcomes, and what forms of direct or indirect intermicrobial interactions lead to them (e.g. working together to provide novel functions vs complementarity of rewards provided to the host by different microbes vs community enhancing a particularly important member of the fungal microbiome).

Diversity–productivity relationships in phyllosphere plant–microbiome interactions

A great deal of research has sought to generalize the relationship between community diversity and productivity, with much of the literature focusing on the relationship between plant diversity and plant productivity (Vermeer & Berendse, 1983; Hector *et al*., 1999; Mittelbach *et al*., 2001; Cadotte *et al*., 2009). These studies often find hump-shaped and positive linear relationship between plant community productivity and plant community taxonomic, functional, or phylogenetic diversity. Leveraging a similar framework, researchers have become increasingly interested in how microbial community diversity could also shape host plant productivity. These studies have investigated belowground fungal microbiomes, often finding a positive linear relationship between belowground fungal diversity and plant productivity (Van Der Heijden *et al.*, 1998, 2006; Vogelsang *et al.*, 2006; Wagg *et al.*, 2011; Koskella, 2020). By contrast, our study – which focused on the phyllosphere fungal microbiome diversity's effects – documented a new quadratic relationship between foliar fungal diversity and total plant biomass. Additional diversity–productivity studies of the phyllosphere will be needed to assess whether the diversity of aboveground fungal communities consistently has different effects on host productivity than belowground communities. However, if common, systemic differences in the effects of phyllosphere vs rhizosphere microbial diversity on host productivity could be related to the naturally lower diversity of phyllosphere fungal microbiomes compared with rhizosphere microbiomes, which has been documented in studies of morning glories as well as other wild plants, crops, and invasive species (Dong *et al.*, 2019; Zhou *et al.*, 2019; Bao *et al.*, 2020). There are several likely explanations for how intermicrobial interactions could underpin the quadratic relationship we found. For instance, some synergistic or antagonistic interactions among fungi in the low-diversity treatment could be disrupted by interactions with other taxa in high-diversity communities (e.g. microbially secreted allelopathic chemicals harming a taxon integral to a synergistic interaction; Afkhami *et al.*, 2020; Brown *et al.*, 2020). Alternatively, the nonlinear relationship could result from the neutral effects of many microbes in high-diversity communities diluting the effects of smaller modules of synergistically or antagonistically interacting microbes on plant productivity. This dilution of nonadditive effects may also result from decreased densities of each taxon in high-diversity communities, which occurs in our experiment and occurs in nature when host resources and space are constrained (McGrady-Steed & Morin, 2000; Hassani *et al.*, 2018). This is because the decreased density of taxa participating in nonadditive interactions could dampen synergistic and antagonistic effects on plant growth. These interactions within the fungal community may be contributing to the nonadditive effects we see in this study leading to plant performance in which the highest diversity treatment plants are comparable to singly inoculated plants.

Conclusions and perspectives on new directions

Overall, this research highlights how interactions within host-associated fungal microbiomes can strongly and nonadditively affect the interaction between plants and microbes as well as the promise of synthetic microbial consortia to understand the complex plant–microbiome interactions that drive host health and productivity. Through this work, we have also identified several areas that would build on and complement our findings. In particular, the frequency of nonadditivity and the complexity of the fungal microbiome necessitate future work that evaluates the mechanisms that underpin nonadditive effects on host productivity and the intermicrobial interactions involved. Differentiating among indirect and direct pathways is intrinsically challenging, especially since multiple mechanisms can simultaneously impact the net effect of the microbiome on the host. To address this challenge, we suggest future work that uses meta-transcriptomic approaches aimed at characterizing changes in microbial functional gene expression between microbes inoculated singly and in more diverse communities as well as co-expression networks analysis to identify shifts in expression that change in tandem between the microbial community members and plant hosts (Palakurty *et al.*, 2018). In addition, meta-transcriptomic studies of communities in the field could be used to form criteria for selection of taxa used in synthetic consortia studies. High-throughput single-cell transcriptomics would further increase the ability to disentangle microbial interactions by allowing the evaluation of intermicrobial co-expression of functional genes (Libault *et al.*, 2010; Ma *et al.*, 2019; Mauger *et al.*, 2021) and could be especially useful for understanding how each microbe and their interactions contribute to the acute synergism events we found for several consortia in this study. Understanding the expression of these functional genes may also provide insight into how species' niche overlaps interact with increasing community diversity to impact plant performance across many functional dimensions simultaneously (e.g. greater functional diversity underlying the effects of greater species diversity on plant productivity; Connor *et al.*, 2017; Afkhami *et al.*, 2020). In our study, we found that all the fungal taxa tested functioned as commensals; however, future work investigating the role of guild or functional diversity would improve understanding of the role of interactions between different microbial functional groups on plant performance. We also advocate for future work investigating how the colonization and populations of fungal taxa change *in planta* in more complex consortia to understand how intermicrobial interactions shape the density and composition of those communities and more closely link changes in microbial community dynamics to nonadditive effects on host plant performance. It could be particularly valuable to focus on population dynamics within consortia that show extreme nonadditivity (e.g. acute synergism) or consortia that include vs exclude taxa identified in our experiment as candidate microbial 'catalysts' for synergisms and antagonisms. In conclusion, our study shows promise for the use of synthetic microbial consortia to understand the complex plant–microbiome interactions that underpinning plant health and microbial community structure and highlights new avenues for future investigations of microbial diversity and intermicrobial interactions.

Acknowledgements

We thank K. Djahed for his assistance with experiment maintenance, and K. Crawford for advice on diversity–productivity study methods. We thank D. Hernandez, K. Kiesewetter, A. Igwe, A. Rawstern, B. Whitlock, and C. Searcy as well as the Wilson, Silveira, and Mueller Labs, editor Öpik and five anonymous reviewers for valuable feedback on this manuscript. We also thank the Environmentally Endangered Lands Program of Miami-Dade County for allowing sample collections and the National Science Foundation for support to M. Afkhami (NSF DEB-1922521 and NSF DEB-2030060) and to B. Almeida (NSF Graduate Research Fellowship Program).

Competing interests

None declared.

Author contributions

BKA and MEA designed the research. BKA and EHT established the experiment and collected the data. BKA analyzed the data with guidance from MEA. BKA, EHT, and MEA wrote the manuscript. BKA and MEA edited and revised the manuscript.

ORCID

Michelle E. Afkhami https://orcid.org/0000-0001-7207-6256
Brianna K. Almeida https://orcid.org/0000-0002-9921-9971

Data availability

Data, metadata, and scripts used in our analyses are available in NCBI Sequence Read Archive (PRJNA874722) and Zenodo (doi: 10.5281/zenodo.7032799).

References

Afkhami ME, Almeida BK, Hernandez DJ, Kiesewetter KN, Revillini DP. 2020. Tripartite mutualisms as models for understanding plant–microbial interactions. *Current Opinion in Plant Biology* **56**: 28–36.

Afkhami ME, Rudgers JA, Stachowicz JJ. 2014. Multiple mutualist effects: conflict and synergy in multispecies mutualisms. *Ecology* **95**: 833–844.

Afkhami ME, Strauss SY. 2016. Native fungal endophytes suppress an exotic dominant and increase plant diversity over small and large spatial scales. *Ecology* **97**: 1159–1169.

Arnold SE, Idrovo MEP, Arias LJL, Belmain SR, Stevenson PC. 2014. Herbivore defence compounds occur in pollen and reduce bumblebee colony fitness. *Journal of Chemical Ecology* **40**: 878–881.

Averill C, Turner B, Finzi A. 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature* **505**: 543–545.

Ballhorn DJ, Schädler M, Elias JD, Millar JA, Kautz S. 2016. Friend or foe-light availability determines the relationship between mycorrhizal fungi, rhizobia and lima bean (*Phaseolus lunatus* L.). *PLoS ONE* **11**: e0154116.

Bao L, Cai W, Cao J, Zhang X, Liu J, Chen H, Wei Y, Zhuang X, Zhuang G, Bai Z. 2020. Microbial community overlap between the phyllosphere and rhizosphere of three plants from Yongxing Island, South China Sea. *Microbiology Open* **9**: e1048.

Barrett LG, Bever JD, Bissett A, Thrall PH. 2015. Partner diversity and identity impacts on plant productivity in Acacia–rhizobial interactions. *Journal of Ecology* **103**: 130–142.

Black WD. 2020. A comparison of several media types and basic techniques used to assess outdoor airborne fungi in Melbourne, Australia. *PLoS ONE* **15**: e0238901.

Brown SP, Grillo MA, Podowski JC, Heath KD. 2020. Soil origin and plant genotype structure distinct microbiome compartments in the model legume *Medicago truncatula*. *Microbiome* **8**: 1–17.

Bybee-Finley KA, Mirsky SB, Ryan MR. 2016. Functional diversity in summer annual grass and legume intercrops in the northeastern United States. *Crop Science* **56**: 2775–2790.

Cadotte MW, Cavender-Bares J, Tilman D, Oakley TH. 2009. Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. *PLoS ONE* **4**: e5695.

Christian N, Herre EA, Clay K. 2019. Foliar endophytic fungi alter patterns of nitrogen uptake and distribution in *Theobroma cacao*. *New Phytologist* **222**: 1573–1583.

Connor EW, Sandy M, Hawkes CV. 2017. Microbial tools in agriculture require an ecological context: stress-dependent non-additive symbiont interactions. *Agronomy Journal* **109**: 917–926.

Cook D, Beaulieu WT, Mott IW, Riet-Correa F, Gardner DR, Grum D, Pfister JA, Clay K, Marcolongo-Pereira C. 2013. Production of the alkaloid swainsonine by a fungal endosymbiont of the Ascomycete order Chaetothyriales in the host Ipomoea carnea. *Journal of Agricultural and Food Chemistry* **61**: 3797–3803.

Crawford KM, Rudgers JA. 2012. Plant species diversity and genetic diversity within a dominant species interactively affect plant community biomass. *Journal of Ecology* **100**: 1512–1521.

Crawford KM, Whitney KD. 2010. Population genetic diversity influences colonization success. *Molecular Ecology* **19**: 1253–1263.

David AS, Seabloom EW, May G. 2016. Plant host species and geographic distance affect the structure of aboveground fungal symbiont communities, and environmental filtering affects belowground communities in a coastal dune ecosystem. *Microbial Ecology* **71**: 912–926.

David AS, Thapa-Magar KB, Menges ES, Searcy CA, Afkhami ME. 2020. Do plant–microbe interactions support the Stress Gradient Hypothesis? *Ecology* **101**: e03081.

De Souza RSC, Armanhi JSL, Arruda P. 2020. From microbiome to traits: designing synthetic microbial communities for improved crop resiliency. *Frontiers in Plant Science* **11**: 1179.

Dolinšek J, Goldschmidt F, Johnson DR. 2016. Synthetic microbial ecology and the dynamic interplay between microbial genotypes. *FEMS Microbiology Reviews* **40**: 961–979.

Dong CJ, Wang LL, Li Q, Shang QM. 2019. Bacterial communities in the rhizosphere, phyllosphere and endosphere of tomato plants. *PLoS ONE* **14**: e0223847.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.

Geiser DM, Taylor JW, Ritchie KB, Smith GW. 1998. Cause of sea fan death in the West Indies. *Nature* **394**: 137–138.

Gohl DM, Vangay P, Garbe J, MacLean A, Hauge A, Becker A, Gould TJ, Clayton JB, Johnson TJ, Hunter R, Knights D. 2016. Systematic improvement of amplicon marker gene methods for increased accuracy in microbiome studies. *Nature Biotechnology* **34**: 942–949.

Gougoulias C, Clark JM, Shaw LJ. 2014. The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. *Journal of the Science of Food and Agriculture* **94**: 2362–2371.

Großkopf T, Soyer OS. 2014. Synthetic microbial communities. *Current Opinion in Microbiology* **18**: 72–77.

Harman G, Khadka R, Doni F, Uphoff N. 2021. Benefits to plant health and productivity from enhancing plant microbial symbionts. *Frontiers in Plant Science* **11**: 610065.

Hartley SE, Gange AC. 2009. Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. *Annual Review of Entomology* **54**: 323–342.

Hassani MA, Durán P, Hacquard S. 2018. Microbial interactions within the plant holobiont. *Microbiome* **6**: 1–17.

Hector A, Schmid B, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J *et al.* 1999. Plant diversity and productivity experiments in European grasslands. *Science* **286**: 1123–1127.

Hicks N, Bulling MT, Solan M, Raffaelli D, White PC, Paterson DM. 2011. Impact of biodiversity-climate futures on primary production and metabolism in a model benthic estuarine system. *BMC Ecology* **11**: 1–8.

Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC *et al.* 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters* **13**: 394–407.

Hori Y, Fujita H, Hiruma K, Narisawa K, Toju H. 2021. Synergistic and offset effects of fungal species combinations on plant performance. *Frontiers in Microbiology* **12**: 713180.

Howard MM, Kao-Kniffin J, Kessler A. 2020. Shifts in plant–microbe interactions over community succession and their effects on plant resistance to herbivores. *New Phytologist* **226**: 1144–1157.

Huangfu C, Zhang L, Hui D. 2022. Density-dependent plant-soil feedbacks of two plant species affected by plant competition. *Science of the Total Environment* **807**: 150908.

Hubbard M, Germida JJ, Vujanovic V. 2014. Fungal endophytes enhance wheat heat and drought tolerance in terms of grain yield and second-generation seed viability. *Journal of Applied Microbiology* **116**: 109–122.

Huston MA. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* **110**: 449–460.

Huston MA, McBride AC. 2002. Evaluating the relative strengths of biotic vs abiotic controls on ecosystem processes. In: Loreau M, Naeem S, Inchausti P, eds. *Biodiversity and ecosystem functioning: synthesis and perspectives.* Oxford, UK: Oxford University Press, 47–60.

Ivic D. 2014. Pathogenicity and potential toxigenicity of seed-borne *Fusarium* species on soybean and pea. *Journal of Plant Pathology* **96**: 541–551.

Jiménez-Gómez I, Valdés-Muñoz G, Moreno-Perlin T, Mouriño-Pérez RR, Sánchez-Carbente MDR, Folch-Mallol JL, Pérez-Llano Y, Gunde-Cimerman N, Sánchez NDC, Batista-García RA. 2020. Haloadaptative responses of *Aspergillus sydowii* to extreme water deprivation: morphology, compatible solutes, and oxidative stress at NaCl saturation. *Journal of Fungi* **6**: 316.

Khare E, Mishra J, Arora NK. 2018. Multifaceted interactions between endophytes and plant: developments and prospects. *Frontiers in Microbiology* **9**: 2732.

Kiers ET, Hutton MG, Denison RF. 2007. Human selection and the relaxation of legume defences against ineffective rhizobia. *Proceedings of the Royal Society B: Biological Sciences* **274**: 3119–3126.

Kiesewetter KN, Afkhami ME. 2021. Microbiome-mediated effects of habitat fragmentation on native plant performance. *New Phytologist* **232**: 1823–1838.

Korenblum E, Dong Y, Szymanski J, Panda S, Jozwiak A, Massalha H, Meir S, Rogachev I, Aharoni A. 2020. Rhizosphere microbiome mediates systemic root metabolite exudation by root-to-root signaling. *Proceedings of the National Academy of Sciences, USA* **117**: 3874–3883.

Koskella B. 2020. The phyllosphere. *Current Biology* **30**: 1143–1146.

Kucht S, Groß J, Hussein Y, Grothe T, Keller U, Basar S, König WA, Steiner U, Leistner E. 2004. Elimination of ergoline alkaloids following treatment of *Ipomoea asarifolia* (Convolvulaceae) with fungicides. *Planta* **219**: 619–625.

Lang AK, LaRue EA, Kivlin SN, Edwards JD, Phillips RP, Gallion J, Kong N, Parker JD, McCormick MK, Domke G *et al.* 2023. Forest structural diversity is linked to soil microbial diversity. *Ecosphere* **14**: 4702.

Larimer AL, Bever JD, Clay K. 2010. The interactive effects of plant microbial symbionts: a review and meta-analysis. *Symbiosis* **51**: 139–148.

Larimer AL, Clay K, Bever JD. 2014. Synergism and context dependency of interactions between arbuscular mycorrhizal fungi and rhizobia with a prairie legume. *Ecology* **95**: 1045–1054.

Li L, Giller P, Peng P, Guo Y, Zhang S, Hu Z, Wang X, Zhao G. 2023. The genetic diversity-productivity effect in wheat cultivar mixtures at multiple levels. *European Journal of Agronomy* **142**: 126676.

Li Z, Tian D, Wang B, Wang J, Wang S, Chen HY, Xu X, Wang C, He N, Niu S. 2019. Microbes drive global soil nitrogen mineralization and availability. *Global Change Biology* **25**: 1078–1088.

Libault M, Farmer A, Brechenmacher L, Drnevich J, Langley RJ, Bilgin DD, Radwan O, Neece DJ, Clough SJ, May GD *et al.* 2010. Complete transcriptome of the soybean root hair cell, a single-cell model, and its alteration in response to *Bradyrhizobium japonicum* infection. *Plant Physiology* **152**: 541–552.

Liu H, Brettell LE. 2019. Plant defense by VOC-induced microbial priming. *Trends in Plant Science* **24**: 187–189.

Long HH, Schmidt DD, Baldwin IT. 2008. Native bacterial endophytes promote host growth in a species-specific manner; phytohormone manipulations do not result in common growth responses. *PLoS ONE* **3**: e2702.

Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, Rio TGD *et al.* 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature* **488**: 86–90.

Ma Q, Bücking H, Gonzalez Hernandez JL, Subramanian S. 2019. Single-cell RNA sequencing of plant-associated bacterial communities. *Frontiers in Microbiology* **10**: 2452.

Malcolm GM, Kuldau GA, Gugino BK, Jiménez-Gasco MDM. 2013. Hidden host plant associations of soilborne fungal pathogens: an ecological perspective. *Phytopathology* **103**: 538–544.

Mauger S, Monard C, Thion C, Vandenkoornhuyse P. 2021. Contribution of single-cell omics to microbial ecology. *Trends in Ecology & Evolution* **37**: 67–78.

McGrady-Steed J, Morin PJ. 2000. Biodiversity, density compensation, and the dynamics of populations and functional groups. *Ecology* **81**: 361–373.

Millar N, Piovia-Scott J, Porter SS. 2023. Impacts of domestication on the rhizobial mutualism of five legumes across a gradient of nitrogen-fertilisation. *Plant and Soil* **491**: 479–499.

Mittelbach GG, Steiner CF, Scheiner SM, Gross KL, Reynolds HL, Waide RB, Willig MR, Dodson SI, Gough L. 2001. What is the observed relationship between species richness and productivity? *Ecology* **82**: 2381–2396.

Morella NM, Weng FCH, Joubert PM, Metcalf CJE, Lindow S, Koskella B. 2020. Successive passaging of a plant-associated microbiome reveals robust habitat and host genotype-dependent selection. *Proceedings of the National Academy of Sciences, USA* **117**: 1148–1159.

Niu B, Paulson JN, Zheng X, Kolter R. 2017. Simplified and representative bacterial community of maize roots. *Proceedings of the National Academy of Sciences, USA* **114**: E2450–E2459.

Okungbowa FI, Shittu HO. 2012. Fusarium wilts: an overview. *Environmental Research Journal* **6**: 83–102.

Omar NH, Mohd M, Nor NMIM, Zakaria L. 2018. Characterization and pathogenicity of *Fusarium* species associated with leaf spot of mango (*Mangifera indica* L.). *Microbial Pathogenesis* **114**: 362–368.

Osborne OG, De-Kayne R, Bidartondo MI, Hutton I, Baker WJ, Turnbull CG, Savolainen V. 2017. Arbuscular mycorrhizal fungi promote coexistence and niche divergence of sympatric palm species on a remote oceanic Island. *New Phytologist* **217**: 1254–1266.

Palakurty SX, Stinchcombe JR, Afkhami ME. 2018. Cooperation and coexpression: how coexpression networks shift in response to multiple mutualists. *Molecular Ecology* **27**: 1860–1873.

Püschel D, Janoušková M, Voříšková A, Gryndlerová H, Vosátka M, Jansa J. 2017. Arbuscular mycorrhiza stimulates biological nitrogen fixation in two *Medicago* spp. Through improved phosphorus acquisition. *Frontiers in Plant Science* **8**: 390.

R Core Team. 2021. *R: a language and environment for statistical computing.* Vienna Austria: R Foundation for Statistical Computing.

Ren CG, Bai YJ, Kong CC, Bian B, Xie ZH. 2016. Synergistic interactions between salt-tolerant rhizobia and arbuscular mycorrhizal fungi on salinity tolerance of *Sesbania cannabina* plants. *Journal of Plant Growth Regulation* **35**: 1098–1107.

Revillini D, David AS, Menges ES, Main KN, Afkhami ME, Searcy CA. 2022. Microbiome-mediated response to pulse fire disturbance outweighs the effects of fire legacy on plant performance. *New Phytologist* **233**: 2071–2082.

Rivera-Varas VV, Freeman TA, Gudmestad NC, Secor GA. 2007. Mycoparasitism of *Helminthosporium solani* by *Acremonium strictum.* *Phytopathology* **97**: 1331–1337.

Roman-Reyna V, Pinili D, Borja FN, Quibod IL, Groen SC, Mulyaningsih ES, Rachmat A, Slamet-Loedin IH, Alexandrov N, Mauleon R *et al.* 2019. The rice leaf microbiome has a conserved community structure controlled by complex host-microbe interactions. *bioRxiv*. doi: 10.1101/615278.

Schardl CL. 1996. Epichloë species: fungal symbionts of grasses. *Annual Review of Phytopathology* **34**: 109–130.

Sharma B, Shukla P. 2020. Designing synthetic microbial communities for effectual bioremediation: a review. *Biocatalysis and Biotransformation* **38**: 405–414.

Šmilauer P, Košnar J, Kotilínek M, Šmilauerová M. 2020. Contrasting effects of host identity, plant community, and local species pool on the composition and colonization levels of arbuscular mycorrhizal fungal community in a temperate grassland. *New Phytologist* **225**: 461–473.

Špaèková I, Lepš J. 2001. Procedure for separating the selection effect from other effects in diversity–productivity relationship. *Ecology Letters* **4**: 585–594.

Subedi SC, Allen P, Vidales R, Sternberg L, Ross M, Afkhami ME. 2022. Salinity legacy: foliar microbiome's history affects mutualist-conferred salinity tolerance. *Ecology* **103**: e3679.

Syed-Ab-Rahman SF, Carvalhais LC, Chua ET, Chung FY, Moyle PM, Eltanahy EG, Schenk PM. 2019. Soil bacterial diffusible and volatile organic compounds inhibit *Phytophthora capsici* and promote plant growth. *Science of the Total Environment* 692: 267–280.

Symstad AJ, Tilman D, Wilson K, Knops JM. 1998. Species loss and ecosystem functioning: effects of species identity and community composition. *Oikos* 81: 389–397.

Thamer S, Schädler M, Bonte D, Ballhorn DJ. 2011. Dual benefit from a belowground symbiosis: nitrogen fixing rhizobia promote growth and defense against a specialist herbivore in a cyanogenic plant. *Plant and Soil* 341: 209–219.

Tilman D, Wedin D, Knops J. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379: 718–720.

Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. 2020. Plant–microbiome interactions: from community assembly to plant health. *Nature Reviews Microbiology* 18: 607–621.

UNITE Community. 2017. *UNITE QIIME release v.01.12.2017.* UNITE Community.

Van Der Heijden MG, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69–72.

Van Der Heijden MG, Streitwolf-Engel R, Riedl R, Siegrist S, Neudecker A, Ineichen K, Boller T, Wiemken A, Sanders IR. 2006. The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. *New Phytologist* 172: 739–752.

Van Wees SC, Van der Ent S, Pieterse CM. 2008. Plant immune responses triggered by beneficial microbes. *Current Opinion in Plant Biology* 11: 443–448.

Vermeer JG, Berendse F. 1983. The relationship between nutrient availability, shoot biomass and species richness in grassland and wetland communities. *Vegetatio* 53: 121–126.

Vogelsang KM, Reynolds HL, Bever JD. 2006. Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytologist* 172: 554–562.

Wagg C, Jansa J, Schmid B, van der Heijden MG. 2011. Belowground biodiversity effects of plant symbionts support aboveground productivity. *Ecology Letters* 14: 1001–1009.

Wężowicz K, Rozpądek P, Turnau K. 2017. Interactions of arbuscular mycorrhizal and endophytic fungi improve seedling survival and growth in post-mining waste. *Mycorrhiza* 27: 499–511.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols: a guide to methods and applications.* New York, NY, USA: Academic Press, 315–322.

Yang T, Adams JM, Shi Y, He JS, Jing X, Chen L, Tedersoo L, Chu H. 2017. Soil fungal diversity in natural grasslands of the Tibetan Plateau: associations with plant diversity and productivity. *New Phytologist* 215: 756–765.

Zhou Q, Zhang X, He R, Wang S, Jiao C, Huang R, He X, Zeng J, Zhao D. 2019. The composition and assembly of bacterial communities across the rhizosphere and phyllosphere compartments of *Phragmites Australis. Diversity* 11: 98.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Relative abundances of taxa from the natural fungal community assigned to functional guilds using FUNGuild.

Fig. S2 Cultures used in the synthetic consortia experiments had a sequence overlap of 98% ± 0.4 with community-wide microbiome data from field-collected host plants.

Fig. S3 Effect of fungal diversity on host plant root mass, shoot mass, height, and the investment in roots vs shoots (i.e. root-to-shoot ratio).

Fig. S4 Plant performance metrics were significantly different between control and diversity treatments in root biomass, shoot biomass, and height as well as on plant allocation between roots and shoots (root-to-shoot ratio).

Fig. S5 Most plants inoculated with experimental consortia performed similarly to plants inoculated with the natural fungal microbiome for both root mass and shoot mass.

Fig. S6 Nonadditive effects of microbial communities on plant performance metrics of root biomass, shoot biomass, and height as well as on plant allocation between roots and shoots (root-to-shoot ratio).

Fig. S7 Percent occurrence of fungal taxa in synergistic and antagonistic consortia for plant traits of root mass, shoot mass, height, and root vs shoot investment.

Fig. S8 Average magnitude of effect on plant growth of nonadditive communities that taxa participated in.

Table S1 Composition of low-diversity communities used to manipulate fungal community diversity.

Table S2 Composition of high-diversity communities used to manipulate fungal community diversity.

Table S3 ANOVA table comparing effects of control and single isolates on plant performance to determine whether fungal taxa are mutualists, commensals, or antagonists in monoculture inoculations.

Table S4 ANOVA table comparing effects of control and low-diversity community inocula.

Table S5 ANOVA table comparing effects of control and high-diversity community inocula.