



Ectomycorrhizal fungal diversity interacts with soil nutrients to predict plant growth despite weak plant-soil feedbacks

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Abstract

Background and aims Plant-soil feedbacks are the result of multiple abiotic and biotic mechanisms. However, few studies have addressed how feedbacks vary based on abiotic context or attempted to identify microbiota responsible for feedbacks. We investigated whether plant-soil feedbacks of an ectomycorrhizal tree (*Quercus macrocarpa*) varied based on soil nutrient status and whether fungal community composition and diversity could explain feedback patterns.

Methods We inoculated *Q. macrocarpa* seedlings with field-sampled soils taken from five soil origins – including heterospecific and conspecific trees and an old field – which were profiled using fungal DNA metabarcoding.

Results There was a positive home vs. away plant-soil feedback, though feedbacks with individual hosts were not significant regardless of fertilization. Still, hosts harbored distinctive fungal communities that were predictive of plant growth. There was a growth promotive effect of ectomycorrhizal OTU diversity that was weakened with fertilization, suggesting context-dependent relationships between plant growth and a guild of fungal mutualists.

Conclusions Our results demonstrate that the host-specific accumulation of functionally important soil microbes is not always sufficient to drive species level plant-soil feedbacks. Our data provide support for a role of ECM fungal diversity in mediating plant growth responses, though it is unclear whether this effect was direct or indirect.

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Introduction

The Janzen-Connell hypothesis proposes that negative distance-dependent and density-dependent effects cause reductions in the growth and survival of seedlings in a localized area surrounding conspecific adults (Connell 1971; Janzen 1970). These effects are postulated to confer a competitive advantage to heterospecific seedlings over conspecifics, thereby increasing alpha diversity (Bever et al. 2012). The Janzen-Connell hypothesis has received support from both simulation and empirical

studies (Comita et al. 2014; Johnson et al. 2012; Mangan et al. 2010; Packer and Clay 2000; Zhu et al. 2015), though other studies suggest that the hypothesis is not a universal rule of community assembly (Hyatt et al. 2003; McCarthy-Neumann and Kobe 2010).

Plant-soil feedback (PSF) theory has extended the Janzen-Connell hypothesis by acknowledging that density dependence can be either positive or negative *and* that density-dependence in either direction can be driven by both mutualists (Bever 2002) and pathogens (Packer and Clay 2004). PSF work has focused on the reciprocal influences exerted between plants and their surrounding soil, wherein plants alter the composition of soil biota and/or the physicochemical properties of the soil, which then affect plant fitness (Bever et al. 1997). Many studies have described positive PSFs that are expected to contribute to local competitive exclusion and the loss of fine-scale diversity (van der Putten et al. 2013). Plant and microbial ecologists are also beginning to recognize the possibility for context-dependent PSFs across abiotic and biotic gradients (Smith-Ramesh and Reynolds 2017). Context-dependent PSFs have already been experimentally documented for light (McCarthy-Neumann and Ibáñez 2013; Smith and Reynolds 2015), soil nitrogen (Valliere and Allen 2016), and drought (Rutten and Gómez-Aparicio 2018; Valliere and Allen 2016) gradients among others.

Abiotically-induced shifts between positive and negative PSFs can be caused by shifts of symbiotic interactions along the mutualism-parasitism continuum (Johnson et al. 1997). A simple cost-benefit analysis predicts that mutualism shifts to parasitism when the cost to one partner becomes greater than the benefit. This shift occurs when either the cost increases or the benefit decreases to a given partner in the symbiosis. Plants often respond less positively to mycorrhizal fungi when nutrients are in excess because the nutrient acquisition services that mycorrhizal fungi provide become reduced or superfluous, though they continue to drain carbon from their plant hosts. For example, numerous studies based on inoculations of plants with pure cultures have shown that mycorrhizal interactions can shift from mutualistic to parasitic with the addition of phosphorous and/or nitrogen (Bethlenfalvay et al. 1983; Bougher et al. 1990; Buwalda and Goh 1982; Graham et al. 1996; Johnson 1993; Kiernan et al. 1983; Koide 1985; Mosse 1973). However, many of the studies demonstrating “mycorrhizal parasitism” have been conducted with arbuscular mycorrhizal (AM) fungi and

there is less support for mycorrhizal parasitism in ectomycorrhizal (ECM) fungi (Karst et al. 2008), though there is still a strong potential for the outcome of such interactions to be nutrient-dependent (Jonsson et al. 2001). Despite the evidence that single-species inoculations with mycorrhizal fungi can have nutrient-dependent effects on plants, there have been fewer studies investigating whether such results are applicable to PSF systems with much more complex fungal communities (Valliere and Allen 2016). Extending the mutualism-parasitism continuum hypothesis to more realistic soil microbial communities that are the norm for PSF experiments could be informative for determining its importance in ecosystems.

Here, we conducted an experiment to understand PSFs acting on the ECM tree species *Quercus macrocarpa* Michx. across two nutrient levels and used amplicon sequencing to investigate the fungal communities of field-sampled soil inocula. We predicted that *Q. macrocarpa* would be subject to more positive PSFs in low nutrient conditions because of its association with ECM fungi (Bennett et al. 2017; Corrales et al. 2016; Teste et al. 2017) and that these PSFs would become more negative with the addition of a general fertilizer that might shift the balance of ECM symbioses along the mutualism-parasitism continuum. We expected that ECM fungi would positively predict plant growth when nutrients are limiting but show less positive, or even negative, associations with growth in fertilized treatments. We included field soils sampled from both ECM and non-ECM tree species as well as from an early successional old field, to incorporate a wide natural range of ECM fungal diversity. We expected that soils taken from ECM hosts would be enriched in ECM fungal taxa and accordingly that there would be less of a growth response to fertilizer in these soils than in the soils with less ECM fungal diversity.

Materials and methods

Soil collection and plant propagation

Soil samples were collected in November of 2015 from Oberlin College’s solar array fields and the adjoining 18.2 ha forest in northeastern Ohio, USA (41°18’12.3”N and 82°13’43.2”W). The solar fields were formerly leased by the college to a local farmer who grew corn and soybeans in rotation until the fields were removed

from cultivation in 2011. Hoffman et al. (2013) estimated the age of the forest surrounding the field to be about 55 years based on tree increment cores. The forest is dominated by *Acer rubrum*, with *A. saccharinum*, *A. saccharum*, *Quercus rubra*, *Q. macrocarpa*, *Q. palustris*, *Q. alba*, *Ulmus americana*, *Tilia americana*, *Fraxinus americana* and *Carya ovata* as associates. Soil was sampled from under 4 different individuals from each tree species (*Quercus macrocarpa*, *Quercus rubra*, *Acer rubrum*, and *Carya ovata*) and from 4 different locations in the adjacent old field for a total of 20 different soil inocula. To ensure that the soil biota sampled represented only the respective species, we sampled soil under parent trees whose trunks were at least 10 m away from heterospecific trees and soil was sampled from four corners of the old field, with the distance to forest edge ranging from 25 m to 45 m. Soil cores (10 cm diameter) were taken to a depth of 15 cm from within a 1 m radius surrounding each tree and composited from multiple soil cores until a volume of about 18 L was reached for each of the 20 sampling locations. Following collection, soils were homogenized and sieved to 6.35 mm to remove large debris and stored at 4 °C for 5 weeks until planting. *Q. macrocarpa* acorns were collected from a single cultivated tree in September of 2015 and were cold-stratified at 4 °C for approximately 3 months. After cold stratification, seeds were surface-sterilized for 1.5 min in 70% ethanol and 3 min in 50% bleach, rinsed in deionized water, and placed on a tray of moist vermiculite in a growth chamber at 24 °C to germinate.

The experiment included 400 seedlings (20 soils × 2 sterilization treatments × 2 fertilization treatments × 5 replicate plants). For control inocula, we sterilized soils by autoclaving twice at 121 °C for 2 h, with 48 h in between the two cycles. Field collected soils were mixed with unsterilized peat moss at a 1:5 ratio (v:v) prior to planting. The use of unsterilized peat moss may have introduced a common microbial community to all pots. Tammi et al. (2001) found minimal ECM colonization of Scots pine when grown in unsterilized peat moss, while Ángeles-Argáiz et al. (2016) found that ECM colonization from peat moss was widespread on seedlings, albeit in low abundance. We planted germinated seeds in 12.7 cm diameter pots filled with prepared potting medium. Due to variation in germling development at planting, we recorded the developmental stage of each germling as either: 1 – radicle barely emerged, no hypocotyl present; 2 – radicle larger than 1 cm, no hypocotyl present; or 3 – radicle and

hypocotyl both present. After sorting germlings into these developmental stage categories, we distributed them uniformly across all treatment combinations, recording the developmental stage of each pot. Pots were arranged on metal shelving units equipped with fluorescent light banks equipped with eight 52 W 4100 k bulbs providing $55.1 \pm 8.9 \mu\text{moles m}^{-2} \text{s}^{-1}$ of light and the positions of the pots were randomized every two weeks. All plants were watered with 100 mL of deionized water every other day until January 25, 2016 when we increased the amount of water to 120 mL for the remainder of the experiment due to minor drought symptoms. Every 8 days we delivered 78.3 mg of Master Nursery© (Vacaville, CA, USA) Water Soluble 18–18–18 fertilizer dissolved in the normal dose of water to each fertilized plant, equating to 14.1 mg N, 6.2 mg P, and 11.7 mg K. This quantity of fertilizer is slightly more than has generally been recommended for containerized production of oak seedlings (Oliet et al. 2011), but less than a dose that has been shown to inhibit mycorrhization (Beckjord et al. 1983). Unfertilized plants did not receive any supplemental nutrients. We allowed seedlings to grow for 45 days and at the end of the growth period, plants were harvested and dry biomass of the roots and shoots was recorded after drying at 80 °C for at least 24 h.

Soil physiochemical analyses and fungal ITS2 gene sequencing

Subsamples of soil were taken shortly after sampling and were sent to Midwest Laboratories (Omaha, NE) for nutrient analysis of the following ten variables: organic matter, phosphorous (weak bray), potassium, magnesium, calcium, sodium, pH, cation exchange capacity (CEC), nitrate nitrogen, and soluble salts using their standard procedures as outlined at <https://midwestlabs.com/>. Fungal community characterization using the ITS2 rRNA gene region was carried out on frozen subsamples shipped to Oak Ridge National Laboratory using the primers and methods described in Cregger et al. (2018).

Data analyses

We used the QIIME pipeline (Caporaso et al. 2010) for sequence read analyses including calculation of alpha and beta diversity and hypothesis testing. After rarefying our dataset to 6591 sequences per sample, we used principal coordinates analysis (PCoA) to visualize effects of soil

origin on fungal community composition with the Bray-Curtis dissimilarity metric. We used the UNITE database to assign taxonomy to OTUs (Kõljalg et al. 2005). We chose not to collapse OTUs with the same taxonomic annotations because taxonomic assignments are often limited by reference sequence availability, which would have led to the possibility that OTUs with the same species level taxonomic annotations originated from different species. This decision resulted in some amount of OTU inflation by taxonomically redundant OTUs (e.g. 74 OTUs were identified as *Kazachstania telluris*), though there was no indication that this introduced bias in estimates of alpha diversity. FUNGuild was used to assign OTUs to ecological guilds, with only guild assignments with a confidence of “probable” or higher being retained (Nguyen et al. 2015). Permutational multivariate analysis of variance (PERMANOVA) in the *vegan* package in R (Anderson 2001; Oksanen et al. 2011; R Core Team 2019) was used to test effects of soil origin and/or specific edaphic factors on fungal communities. MANOVA was used to test differences in abiotic properties among soil origins.

Because only 9 of the 400 plants died during the experiment and there was no apparent pattern in the mortality, we removed plants that did not survive until the end of the growth trial from our dataset. We conducted a quantitative comparison of three methods of calculating PSF to determine which had the best statistical properties and present a description of these tests in supplemental file 2. Ultimately, we settled on a method that involved 1) calculating an index Eff_{micro} of microbial effects on plant growth (i.e. the effect of autoclaving soil inoculum) for each plant in unsterilized soil and 2) fitting mixed models to Eff_{micro} as a statistical test of PSF, taking the model coefficients for all away soils (with the values in the *Q. macrocarpa* home soils set as the model’s intercept) as a measure of PSF. We calculated Eff_{micro} for each plant grown in *unsterilized* soil using a modified version of an equation from Pernilla Brinkman et al. (2010). For each plant n that was inoculated with unsterilized inoculum from soil sample i with fertilization treatment f , we calculated the effect of microbiota as,

$$Eff_{micro} = \left(\left(\frac{growth_n}{growth_{i,f,ster}} \right) - 1 \right) * 100$$

Note that $growth_n$ refers to the growth metric (biomass or root/shoot) of a single plant grown in unsterilized soil, whereas $growth_{i,f,ster}$ refers to the mean growth of all replicate plants grown in sterilized soil sample i

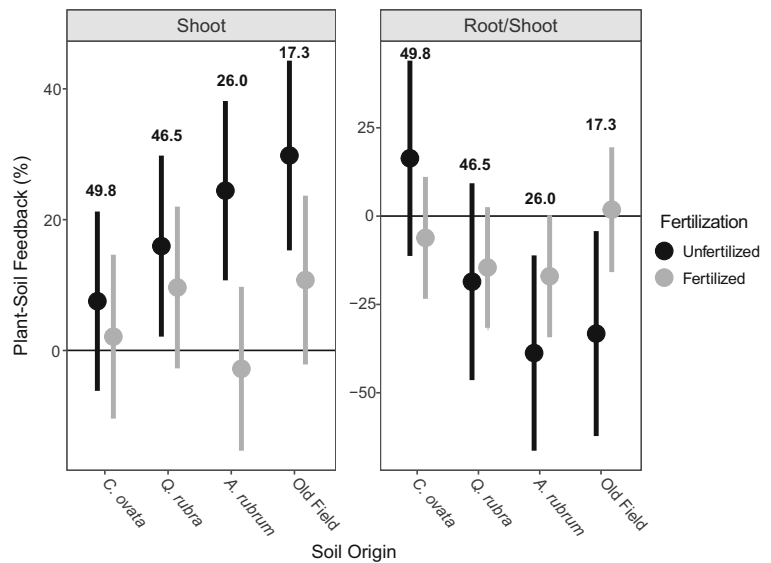
with fertilization status f . Positive values of Eff_{micro} indicate a positive effect of microbiota on the growth parameter, whereas negative values indicate a negative effect of microbiota on the growth parameter. Using Eff_{micro} in mixed models rather than raw biomass data is meant to test the effect of *biotically* mediated feedbacks instead of *abiotically* mediated feedbacks. We assessed whether there was a gross effect of soil microbiota on plant growth for each soil origin by testing deviation of Eff_{micro} values from 0 using mixed models with REML estimation as implemented in the *lme4* package in R (Bates et al. 2014). We also used mixed models to test whether there was an effect of fungal OTU richness or community composition on Eff_{micro} . We used the first two axes of the PCoA of fungal community composition as independent variables in the latter models. The significance of effects in all mixed models was tested by analysis of variance (ANOVA) with type II sum of squares. All plots were generated in R with the *ggplot2* package (Wickham 2016).

Results

Plant-soil feedback and growth

There was a marginally significant positive home vs. away PSF on shoot biomass (see Fig. 1; $P = 0.07$), though there were not any significant plant-soil feedbacks for individual soil origins. There were no significant effects of fertilization on PSF ($P = 0.22-0.34$), though unfertilized *Q. macrocarpa* soils did display a weak positive trend in shoot biomass PSFs with all other soil origins, a pattern that disappeared with fertilization, in agreement with our hypothesis (see Fig. 1). PSFs were more strongly repressed by fertilization for non-ECM hosts than for ECM hosts, though these interactions were not significant (see Fig. 1). Across all soil origins, soil sterilization positively affected shoot biomass (see Table S1; $Eff_{micro} = -16.2\% \pm 3.2\%$, $P < 0.0001$), though effects of soil sterilization on root/shoot ratio ($Eff_{micro} = 8.8\% \pm 5.4\%$, $P = 0.099$) were inconsistent between origins and not globally significant (see Fig. 2). Post-hoc tests found only a few significant effects of soil sterilization on individual soil origin:fertilization groups for either growth metric (see Fig. 2). In light of the highly significant global effects of soil sterilization on shoot biomass, the lack of consistently significant post-hoc tests shows that soil microbial

Fig. 1 Measures of plant-soil feedback are derived as coefficients of mixed models using soil origin to predict Eff_{micro} of shoot biomass and root/shoot ratio. The plant-soil feedback metric is the estimate of the difference in Eff_{micro} between each of the away origins and the *Q. macrocarpa* “home” soils. The numbers indicate mean ECM OTU diversity for each soil origin. Error bars represent standard errors. No individual PSF was significant, though there was a marginally significant home vs. away effect ($P=0.07$)



effects were not strong enough to be detectable within individual treatment groups but were consistently

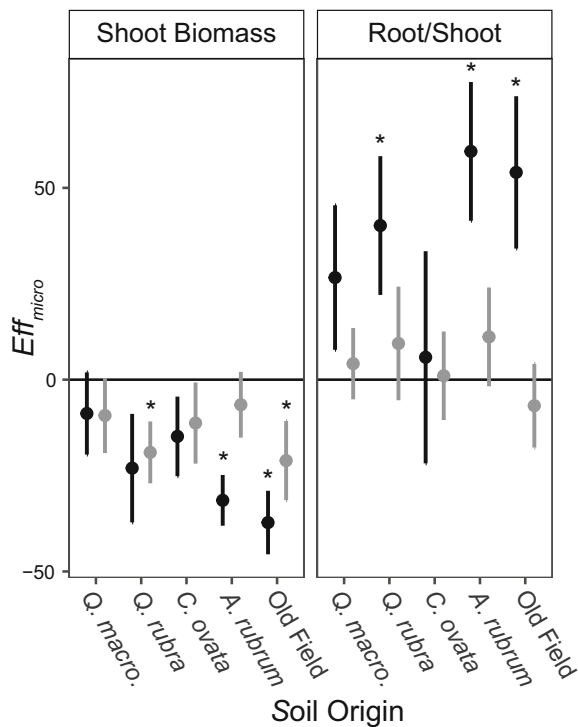


Fig. 2 The effect of microbiota (Eff_{micro}) on shoot biomass and root/shoot is plotted for each soil origin in unfertilized (black bars) and fertilized (grey bars) treatments. Significant effects ($P < 0.05$) of microbiota on growth are indicated by asterisks for individual treatment groups. Error bars represent standard errors. Microbial effects were made weaker by fertilization, particularly for root/shoot

negative across treatment groups. Conservatively, we can conclude that the microbiota from the old field soils strongly inhibited plant growth, with generally significant negative effects on shoot biomass. (fert $Eff_{micro} = -21.1\% \pm 10.3\%*$; unfert $Eff_{micro} = -37.2\% \pm 8.3\%***$). Indeed, the old field soils accounted for 3 out of 7 of the significant post-hoc tests on individual soil origin:fertilization groups.

Fertilization increased shoot biomass ($P < 0.0001$), but decreased the root/shoot ratio ($P < 0.0001$). Fertilization also reduced the magnitude of negative effects of soil microbiota on shoot biomass (see Tables S2, S4; $P = 0.053$) and of positive effects of soil microbiota on root/shoot ratio (see Tables S3, S4; $P = 0.0002$). Post-hoc tests found limited nutrient-induced shifts in microbiota effects for individual soil origins (see Fig. 2), possibly due to insufficient sample size. These results demonstrate that the effects of soil microbiota on shoot biomass and root/shoot ratio are greatest under conditions of low nutrient availability, consistent with our postulate that changes along the mutualism-parasitism continuum may account for PSFs.

Fungal community composition and diversity

Mean OTU richness per sample was 431.6 ± 98.5 . This seemingly high level of diversity was partially driven by redundant OTUs wherein multiple OTUs were given the same taxonomic assignment. Although *Q. rubra* soils tended to be slightly less diverse than other soils, we did

not observe any significant differences in total OTU richness among origins (see Table 1; ANOVA, $R^2 = 0.37$, $P = 0.112$). Although diversity was similar between soil origins, we found that fungal community composition differed between origins (see Fig. 3; $R^2 = 0.31$, $P = 0.001$). This effect was still significant even when the first two axes of an ordination of edaphic properties were included as covariates, though with somewhat less explanatory power ($R^2 = 0.25$, $P = 0.001$), showing that soil origin explains additional variation in fungal communities beyond what can be explained by the soil chemistry variables measured. PCoA (see Fig. 3) and pairwise PERMANOVA (see Table S5) of fungal community composition showed that soils from the old field tended to cluster apart from the four forest soils. Three of the four *A. rubrum* soils formed a distinct cluster apart from the three ECM hosts, with the fourth *A. rubrum* soil placed amongst the ECM hosts, though there was little statistical support for any distinction between the ECM host soils and the *A. rubrum* soils (see Fig. 3, Table S5). Splitting our dataset into separate fungal guilds showed that soil origin was a significant predictor of the composition of each guild (see Fig. 3; $P < 0.001$). Pairwise tests on subsets of the data split by fungal guild were similar to tests on the full dataset for the AM, pathogenic, and saprotrophic guilds, with highly significant effects of soil origin on fungal community composition that were largely driven by differentiation between the old field soils and the four tree soil origins. Pairwise tests showed that ECM fungal communities of *A. rubrum* soils were significantly different from *Q. macrocarpa* and *Q. rubra* soils (see Fig. 3, Table S5), though this effect may be explained by the greater dispersion in *A. rubrum* ECM communities which can result in significant PERMANOVA results (Anderson 2001).

Individual edaphic factors were also significant predictors of fungal community composition (see Fig. 3). We found significant effects of potassium, organic matter, pH, cation exchange capacity, magnesium, soluble salts, and calcium on fungal community composition (listed in decreasing order of R^2 ; see Table S6). However, soil origin explained a greater portion of variation in soil fungal community composition ($R^2 = 0.31$, $P = 0.001$) than did any of the individual abiotic variables (see Fig. 3; $R^2 = 0.06$ – 0.15 , $P = 0.001$ – 0.30). We also found differences in abiotic edaphic properties between soils of different origins (MANOVA, $P = 0.011$).

Table 1 Bonferroni corrected P -values of pairwise t-tests measuring differences in OTU richness between soil origins for all guilds (a), AM (b), ECM (c), and pathogens (d). Pairwise tests of saprotroph OTU richness were also conducted, but never approached significance ($P \geq 0.43$). Numbers in parentheses indicate the mean \pm SD

	<i>A. rubrum</i> (442 \pm 34)	<i>C. ovata</i> (402 \pm 92)	Old Field (514 \pm 123)	<i>Q. macro</i> (460 \pm 61)	c)	<i>A. rubrum</i> (26 \pm 16)	<i>C. ovata</i> (50 \pm 10)	Old Field (17 \pm 5)	<i>Q. macro</i> (55 \pm 6)
a)									
<i>C. ovata</i> (402 \pm 92)	1	–	–	–	<i>C. ovata</i> (50 \pm 10)	0.03	–	–	–
Old Field (514 \pm 123)	1	0.92	–	–	Old Field (17 \pm 5)	1	0.002	–	–
<i>Q. macro</i> (460 \pm 61)	1	1	1	–	<i>Q. macro</i> (55 \pm 6)	0.007	1	5.20E-04	–
<i>Q. rubra</i> (340 \pm 102)	1	1	0.14	0.73	<i>Q. rubra</i> (47 \pm 8)	0.081	1	0.006	1
b)					d)				
<i>A. rubrum</i> (26 \pm 5)	1	1	1	1	<i>A. rubrum</i> (24 \pm 8)	1	1	1	1
<i>C. ovata</i> (9 \pm 4)	0.58	–	–	–	<i>C. ovata</i> (25 \pm 9)	1	–	–	–
Old Field (75 \pm 23)	2.70E-04	8.80E-06	–	–	Old Field (36 \pm 12)	0.611	0.827	–	–
<i>Q. macro</i> (14 \pm 4)	1	1	2.10E-05	–	<i>Q. macro</i> (24 \pm 6)	1	1	0.659	–
<i>Q. rubra</i> (12 \pm 10)	1	1	1.40E-05	1	<i>Q. rubra</i> (16 \pm 8)	1	1	0.048	1
					<i>Q. macro</i> (75 \pm 23)	1	1	1	1
					<i>A. rubrum</i> (9 \pm 4)	1	1	1	1
					<i>C. ovata</i> (9 \pm 4)	1	1	1	1
					Old Field (75 \pm 23)	1	1	1	1
					<i>Q. macro</i> (14 \pm 4)	1	1	1	1
					<i>Q. rubra</i> (12 \pm 10)	1	1	1	1
					<i>A. rubrum</i> (26 \pm 5)	1	1	1	1
					<i>C. ovata</i> (9 \pm 4)	1	1	1	1
					Old Field (75 \pm 23)	1	1	1	1
					<i>Q. macro</i> (14 \pm 4)	1	1	1	1
					<i>Q. rubra</i> (12 \pm 10)	1	1	1	1
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					<i>C. ovata</i> (9 \pm 4)	1	1	1	1
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					<i>Q. rubra</i> (12 \pm 10)	1	1	1	1
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					<i>Q. macro</i> (14 \pm 4)	1	1	1	1
					<i>Q. rubra</i> (12 \pm 10)	1	1	1	1
					<i>A. rubrum</i> (26 \pm 5)	1	1	1	1
					<i>C. ovata</i> (9 \pm 4)	1	1	1	1
					Old Field (75 \pm 23)	1	1	1	1
					<i>Q. macro</i> (14 \pm 4)	1	1	1	1
					<i>Q. rubra</i> (12 \pm 10)	1	1	1	1
					<i>A. rubrum</i> (26 \pm 5)	1	1	1	1
					<i>C. ovata</i> (9 \pm 4)	1	1	1	1
					Old Field (75 \pm 23)	1	1	1	1
					<i>Q. macro</i> (14 \pm 4)	1	1	1	1
					<i>Q. rubra</i> (12 \pm 10)	1	1	1	1
					<i>A. rubrum</i> (26 \pm 5)	1	1	1	1
					<i>C. ovata</i> (9 \pm 4)	1	1	1	1
					Old Field (75 \pm 23)	1	1	1	1
					<i>Q. macro</i> (14 \pm 4)	1	1	1	1
					<i>Q. rubra</i> (12 \pm 10)	1	1	1	1
					<i>A. rubrum</i> (26 \pm 5)	1	1	1	1
					<i>C. ovata</i> (9 \pm 4)	1	1	1	1
					Old Field (75 \pm 23)	1	1	1	1
					<i>Q. macro</i> (14 \pm 4)	1	1	1	1
					<i>Q. rubra</i> (12 \pm 10)	1	1	1	1
					<i>A. rubrum</i> (26 \pm 5)	1	1	1	1
					<i>C. ovata</i> (9 \pm 4)	1	1	1	1
					Old Field (75 \pm 23)	1	1	1	1
					<i>Q. macro</i> (14 \pm 4)	1	1	1	1
					<i>Q. rubra</i> (12 \pm 10)	1	1	1	1
					<i>A. rubrum</i> (26 \pm 5)	1	1	1	1
					<i>C. ovata</i> (9 \pm 4)	1	1	1	1
					Old Field (75 \pm 23)	1	1	1	1
					<i>Q. macro</i> (14 \pm 4)	1	1	1	1
					<i>Q. rubra</i> (12 \pm 10)	1	1	1	1
					<i>A. rubrum</i> (26 \pm 5)	1	1	1	1
					<i>C. ovata</i> (9 \pm 4)	1	1	1	1
					Old Field (75 \pm 23)	1	1	1	1
					<i>Q. macro</i> (14 \pm 4)	1	1	1	1
					<i>Q. rubra</i> (12 \pm 10)	1	1	1	1
					<i>A. rubrum</i> (26 \pm				

Although we did not find differences in total OTU richness between origins, we identified significant variation in the diversity of individual fungal guilds (see Table 1). A wide natural range of ECM fungal diversity was present in sampled soils. Old field soils had the lowest ECM OTU diversity (24.25 ± 5.25), while the *Q. macrocarpa* soils had the highest (58.0 ± 4.55 ; see Table 1). Likewise, ANOVA ($R^2 = 0.68$, $P < 0.005$) and pairwise t-tests (see Table 1) confirmed that soils sampled from the ECM hosts generally had a higher richness of ECM fungi than did the *A. rubrum* or old field soils, which had similar ECM diversity. This effect was robust to the inclusion of the edaphic factor ordination axes as covariates ($P = 0.005$). We also found highly significant differences between origins in AM OTU richness (see Table 1; ANOVA, $R^2 = 0.83$, $P < 0.001$), with the old field soils having a much higher diversity of AM fungi than any other soil (75.25 ± 23.19 compared to 15.00 ± 8.70), which was likely due to the greater abundance and diversity of AM plant hosts. Similarly to the case of ECM fungi, this effect was still significant when edaphic factors were included as covariates ($P = 0.03$). *A. rubrum* soils trended toward a slightly higher richness of AM fungi than soils from ECM hosts, though these effects were not significant in pairwise comparisons (see Table 1). Old field soils had a higher richness of plant pathogenic OTUs than did any of the

forest soils, though the effect was only marginally significant (see Table 1; ANOVA, $R^2 = 0.43$, $P = 0.065$). We did not find any significant effects of soil origin on saprotroph OTU richness (ANOVA, $R^2 = 0.31$, $P = 0.21$).

Correlating plant growth with fungal community composition and diversity

Models testing effects of overall fungal community composition on Eff_{micro} found a main effect of fungal PC1 for shoot biomass ($P = 0.026$) and an interactive effect of fungal PC1 with fertilization for root/shoot ratio (see Fig. 3, Table S7; $P = 0.036$). We also repeated these tests using ECM fungal community composition (instead of overall community composition) to determine whether the composition of this single functional group could predict plant growth (see Fig. 3, Table S10). ECM PC1 had a strongly significant effect on Eff_{micro} of root/shoot ratio ($P = 0.0001$) and a marginally significant effect for shoot biomass ($P = 0.052$). Furthermore, we found an interactive effect between ECM PC1 and fertilization on Eff_{micro} of root/shoot ratio (see Fig. 3; $P = 0.03$), suggesting nutrient dependent effects of ECM fungal community composition on plant biomass allocation. Total fungal OTU diversity negatively correlated with Eff_{micro} of shoot biomass ($P = 0.065$), but not root/

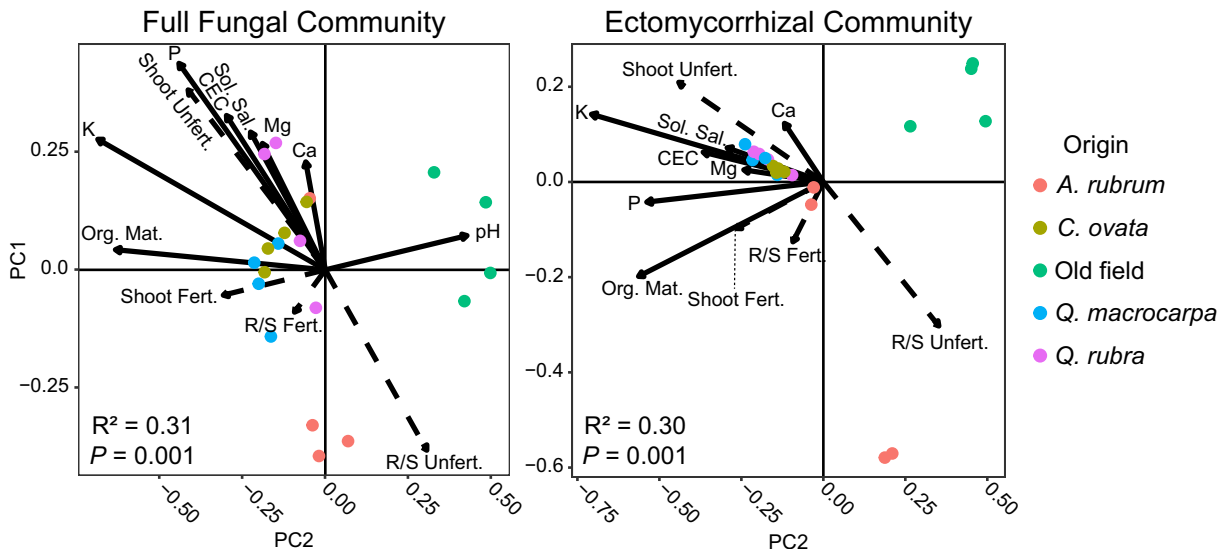


Fig. 3 Principal coordinate analyses of full fungal and ECM community composition data calculated with Bray-Curtis dissimilarity. Edaphic properties that significantly predict fungal community composition (PERMANOVA, $p < 0.05$) are projected as vectors with solid lines. Vectors with dashed lines represent mean

Eff_{micro} values for root/shoot (R/S) and shoot growth under fertilized or unfertilized treatments and are displayed regardless of significance. R^2 and P -values are from PERMANOVA using soil origin to predict fungal community composition

shoot ratio (see Tables S8, S9; $P = 0.146$), regardless of fertilization. AM OTU diversity was a negative predictor of Eff_{micro} of shoot biomass ($P = 0.01$), regardless of fertilization status (see Tables S8, S9). Pathogen OTU diversity was a weak, negative predictor of Eff_{micro} of shoot biomass ($P = 0.21$). The negative correlation between AM diversity and shoot Eff_{micro} were largely driven by the old field soils, which had relatively more negative effects on plant growth and a markedly higher diversity of AM OTUs than other soil origins. Indeed, when old field soils were omitted from analyses, there was no significant effects of AM OTU diversity on shoot Eff_{micro} . As a guild, ECM fungi were unique from all other guilds in that they had generally positive effects on Eff_{micro} , with a marginally significant main effect observed on shoot biomass ($P = 0.053$), though a significant interaction term ($P = 0.049$) indicates that this effect was weakened by fertilization (see Fig. 4, Tables S8, S9). Interactive effects with fertilization on Eff_{micro} of root/shoot ratio were observed for both AM and ECM OTU diversity, though in opposite directions; ECM diversity effects on Eff_{micro} of root/shoot changed

from strongly negative to weakly positive with fertilization whereas AM effects changed from moderately positive to weakly negative. AM and ECM OTU diversity were negatively correlated ($P = 0.001$). Despite this collinearity, when both AM and ECM OTU richness were included in a single model, there was still a marginally significant effect of ECM diversity on Eff_{micro} of root/shoot ratio ($P = 0.051$), while the effect of AM diversity disappeared ($P = 0.419$). Similarly, inclusion of both AM and ECM diversity in a model predicting Eff_{micro} of shoot biomass pointed to a greater effect of ECM diversity ($P = 0.062$) than AM diversity ($P = 0.13$). In contrast, the ratio of ECM:AM OTU diversity provided little explanatory power ($P = 0.46-0.56$).

Discussion

Based on existing knowledge of ECM ecology (Dickie et al. 2014) and the mutualism-parasitism continuum, we hypothesized that *Q. macrocarpa* seedlings would experience positive PSFs when unfertilized and

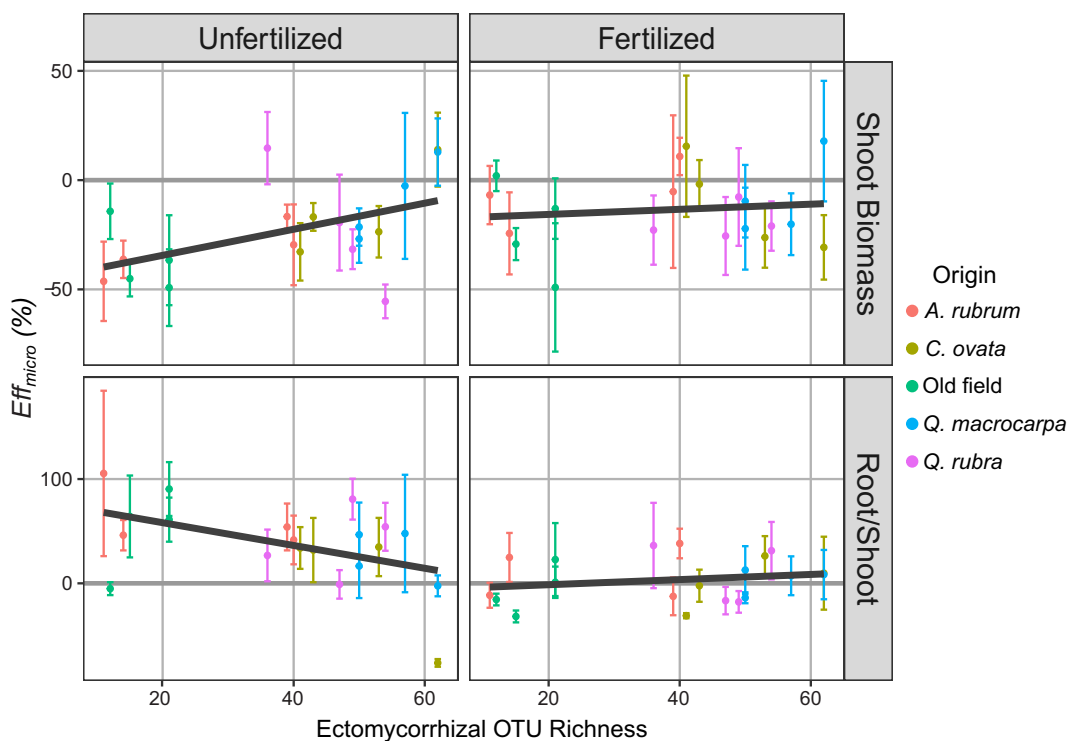


Fig. 4 Relationships between Eff_{micro} of plant growth parameters and ECM OTU richness. Each point represents the mean Eff_{micro} in a single soil with error bars representing the standard error. The interaction term was significant for shoot biomass ($p = 0.049$) and

root/shoot ($p < 0.005$). Note: for ease of interpretation, regression lines are based on standard linear models, rather than on the mixed models used for statistical inference

negative PSFs when fertilized. Although we found generally positive though weak PSFs, there were not significant microbially-mediated feedbacks with specific soil origins (see Fig. 1), likely suggesting a lack of statistical power. We also found strong evidence that 1) interactions between fertilization and soil microbiota determine plant growth responses and allocation (see Figs. 2, 3, 4), and that 2) individual plant hosts harbored distinct assemblages of fungi (see Fig. 3, Tables S5, S7). Although we did not find evidence of microbially-mediated PSFs between any individual away soils and the *Q. macrocarpa* soils, these two main findings are preconditions for the emergence of nutrient-dependent, microbially-mediated PSFs. Soils from under AM plants that had lower ECM OTU richness (i.e. *A. rubrum* and old field) were associated with positive shoot feedbacks that disappeared with fertilization whereas soils from ECM hosts that had greater ECM OTU richness (i.e. *Q. macrocarpa* and *C. ovata*) had generally weak feedbacks regardless of fertilization (see Fig. 1). This pattern suggests that in some cases feedbacks may be structured by the mycorrhizal type of plant hosts rather than by host species and that there may be a relatively greater fitness benefit for seeds of ECM species to be dispersed under ECM plants than under AM plants. Fertilization mitigated the negative effects of inoculation with *A. rubrum* and old field soils on shoot biomass but had little effect on shoot biomass responses to inoculation with any of the ECM host soils (see Fig. 2). This observation, along with our finding of a nutrient-dependent effect of ECM OTU diversity on Eff_{micro} of shoot biomass and root/shoot ratio (see Fig. 4) suggests that the relative dominance of ECM fungi in soil can influence plant responses to fertilization. We find further support for nutrient-dependent effects of ECM fungi from the interactive effect of ECM fungal community composition and fertilization on root/shoot ratio (see Fig. 3, Table S10). Interactions between ECM diversity and fertilization on plant growth were documented in a previous study that grew *Betula pendula* and *Pinus sylvestris* in synthetic ECM communities ranging from one to eight species at two levels of soil fertility and found that effects of ECM diversity on plant biomass were positive or neutral at low soil fertility and became neutral or negative at high soil fertility (Jonsson et al. 2001). Our study demonstrates a similar pattern in a natural gradient of ECM diversity which contained greater ECM richness and included naturally co-occurring microbial taxa. Baxter and Dighton (2005)

found positive effects of ECM diversity when phosphorus was provided as an organic source, but not when an equal quantity of inorganic phosphate was provided. This finding suggests that our results may have been different if nutrients were added in a recalcitrant form rather than the soluble inorganic fertilizer that we used. Our results provide only weak evidence for a direct role of plant pathogens in determining plant growth responses (see Tables S8, S9) as has been observed in other systems (Liu et al. 2012; Mills and Bever 1998; Packer and Clay 2000), though past work has indeed suggested that ECM systems may be less affected by pathogens than AM systems (Bennett et al. 2017; Teste et al. 2017).

Our findings that ECM fungi have a role in determining plant growth responses to nutrient status are correlative and do not establish a mechanism for the growth response. It is plausible that guilds of microbes other than ECM fungi may underly the observed correlations between plant growth and ECM OTU diversity. Indeed, we found that ECM and AM fungal diversity were strongly negatively correlated, confounding the two variables in our analyses, though model selection suggested an effect of ECM fungal diversity on plant growth independent of AM fungal diversity. Although our hypothesis was focused on the potential role of ECM fungi in structuring growth responses, our results suggest that the categorically different ways that soils from AM and ECM hosts affect seedling growth may be due to the relative balance between ECM and AM fungal richness, rather than the diversity of either guild individually.

Although *Quercus* species are considered obligately ECM, they can also form AM associations (Dickie et al. 2001) which can elicit positive growth responses (Egerton-Warburton and Allen 2001). However, we generally observed negative effects of AM OTU diversity and positive effects of ECM OTU diversity on plant growth, providing no support for a growth-promotive role of AM fungi in our experiment. However, AM fungi can affect plant growth in ways beyond mycorrhizal facilitation through indirect mechanisms. It is plausible that interactions between ECM or AM fungi and other microbial guilds structured initial soil microbial communities. For example, competition between mycorrhizal fungi of different types (Knoblochová et al. 2017) and between mycorrhizal fungi and saprotrophs (Bödeker et al. 2016; McHugh and Gehring 2006) are known to contribute to microbial community structure.

Initial soil inoculum communities may also have been structured by facilitative effects such as the recruitment of unique assemblages of bacteria on the surfaces of hyphae (Uroz et al. 2007), which could result in a positive correlation between bacterial diversity and ECM fungal diversity by increasing the variety of hyphosphere niches for bacterial colonization. The potential for belowground inter-guild interactions make it difficult to disentangle the roles of fungi and bacteria in determining plant growth responses. Though adding a layer of complexity to the interpretation of our results, these interactions offer potential mechanistic explanations, albeit indirect, by which mycorrhizal fungal guilds can affect plant growth.

The balance between AM and ECM fungal dominance in our soils could be indicative of variation in a wider suite of microbially mediated processes that may have consequences for plant growth and PSF. Phillips et al. (2013) proposed the mycorrhizal-associated nutrient economy framework, which suggests that the abundance of AM and ECM in a stand may provide an integrated index of biogeochemical transformations related to C and N cycling. AM plants are hypothesized to rely on an “inorganic nutrient economy” dominated by saprotrophs and characterized by fast rates of mineralization and nitrification. In contrast, ECM plants are thought to rely on an “organic nutrient economy” with higher proportions of nutrients retained in organic form. Sequence-based datasets such as ours may provide a similar index that is predictive of the abundances and/or activities of microbial guilds beyond those targeted by the primer set.

Our amplicon sequencing dataset should be interpreted as a catalog of the potential fungal species pool that may have established throughout our growth period and may not precisely represent species active during the growth period. Soil homogenization is known to disrupt mycelial networks and favor ruderal fungal species that are present in the resistant propagule bank (Glassman et al. 2015; Taylor and Bruns 1999), likely excluding late-successional taxa such as members of the Russulaceae (Avis et al. 2017) which are important ECM species in temperate North American oak woodlands (Dickie et al. 2009; Walker et al. 2005). The establishment of field-sampled microbiota may have been further limited by the peat growing medium, which has a much higher organic matter content and C:N ratio than most forest soils. Although we did not take data on the colonization of roots by ECM fungi or

the establishment of free-living fungi, past comparisons of greenhouse bioassay fungal communities with field fungal communities suggest that disturbance-adapted ECM fungal taxa dominate in greenhouse trials such as ours that involve soil sieving (Baar et al. 1999; Duhamel et al. 2019). Accordingly, our findings may be more relevant to PSFs following stand-replacing disturbance than to PSFs following mortality of individual trees within a stand.

While limited by the scope of this study as discussed above, our use of a sequence-based characterization of soil fungal communities was able to provide additional data on soil microbial properties beyond that which can be gleaned from a categorical “plant host” explanatory variable. For example, we found a substantial richness of ECM taxa in all AM soils, and substantial AM richness in all ECM soils, with all ECM soils having comparable AM OTU diversity to *A. rubrum* soils (see Table 1). Two of the *A. rubrum* soils had noticeably higher diversities of ECM fungi than other AM host soils. These displacements of mycorrhizal inocula would not be addressed by standard PSF studies that do not describe microbial community composition. Although these observations were not entirely predictable based on our delineation of tree hosts, they perhaps make sense based on knowledge of tree rooting distributions and fungal spore dispersal. Occurrence of AM and ECM species in the soil inocula originating from mismatched host soils could be due to overlap in root distributions, as roots can extend up to 22 m away from the boles of trees (Jones et al. 2011), substantially further than the minimum 10 m distance from heterospecific trees used for sampling in our study. However, root distributions cannot explain the presence of ECM sequences in the old field soils which were sampled much further than 22 m from the forest edge and are likely derived from spore dispersal (Dickie and Reich 2005; Glassman et al. 2015; Peay and Bruns 2014; Peay et al. 2012). It is worth noting that some fungi are rarely successful colonizers of seedlings from spores and depend heavily on extraradical hyphal colonization from established trees or root fragments (Jones et al. 2003; Taylor and Bruns 1999).

Our observation that plant growth is significantly affected by soil fungal community diversity, but not by soil origin, suggests that initial microbial community composition may have important effects on plant growth even when there are not observable species level PSFs. The lack of significant effects of soil origin also may have arisen from our replication scheme, which maintained the independence of individual soil samples

throughout the growth period. PSF studies that employ individual (rather than mixed) field-sampled soils as the experimental units often have lower precision than experiments using mixed soils, though they provide a more robust estimation of biologically meaningful variation in plant growth responses (Rinella and Reinhart 2018; Smith-Ramesh and Reynolds 2017). When the 16 away soils were pooled, PSFs approached the cutoff for significance, which indicates that higher sample sizes may be required to detect significant PSFs in field-sampled microbial communities, which are frequently highly variable even within single sites (Davison et al. 2012; Štursová et al. 2016). Nevertheless, our data suggest that PSFs are poorly predictive of *Q. macrocarpa* early growth responses to microbes and soil nutrient status. The magnitude of our results may have been limited by the length of the growth period which was short relative to the lifespan of trees. We also stress that the results of our study pertain to a single *Q. macrocarpa* seed family, which may not be representative of the species or population as a whole, due to the widely recognized importance of genetic variation in determining the outcome of mycorrhizal associations (Cline and Patrick Reid 1982; Leski et al. 2010; Tagu et al. 2001).

Our study supports past work demonstrating positive relationships between mycorrhizal fungal diversity and plant productivity (Jonsson et al. 2001; Maherali and Klironomos 2007; van Der Heijden et al. 1998; Wagg et al. 2011), though we found a generally negative effect of *overall fungal diversity* on plant growth metrics. Many questions remain about the functional significance of ECM fungal diversity. Further studies might collect data on extracellular enzyme activities on ECM root tips and employ stable isotope probing to determine the extent to which ECM fungi exhibit functional redundancy or complementarity in their access to organic and inorganic nutrient substrates under varying soil nutrient environments (Jones et al. 2010). Such data could provide a mechanistic understanding of diversity-productivity relationships in ECM systems and provide insight into the contribution of individual fungal taxa to emergent ecosystem processes.

Incorporating environmental gradients into PSF work is increasingly important considering the progression of ongoing global environmental change (van der Putten et al. 2016). Our findings regarding the disruption of ECM symbioses by nutrient enrichment are particularly relevant because of the global crises of nutrient runoff and aerial nitrogen deposition that have affected most if not all of earth's ecosystems (Vitousek et al. 1997). Anthropogenic

nitrogen deposition has already been shown to decrease the richness and colonization of ECM fungi on a global scale (Arnolds 1991; Cox et al. 2010; Treseder 2004; van Strien et al. 2017). Our study suggests that nutrient enrichment also disrupts the ability of ECM fungi to promote plant growth. Although the power of our findings to predict spatial patterns of tree community composition in the field is limited because of the lack of significant species level PSFs, further work investigating how nutrient enrichment alters plant-microbe interactions has the power to predict responses of forest composition to changes in soil nutrient status. Changes in forest composition from dominance by ECM trees to AM trees has further relevance in the context of climate change because of recent work suggesting that such changes may result in the loss of soil carbon stocks and a substantial contribution to global greenhouse gas emissions (Averill et al. 2018). Continued work assessing the role of the abiotic environment in determining the outcome of plant-microbe interactions will be imperative for as long as the abiotic environment continues to be rapidly modified by humans on a global scale.

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Data Availability The sequence datasets generated during the current study are available at the NCBI Sequence Read Archive under the BioProject ID: PRJNA486026.

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