

RESEARCH ARTICLE

Non-symbiotic soil microbes are more strongly influenced by altered tree biodiversity than arbuscular mycorrhizal fungi during initial forest establishment

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One sentence summary: We found that, 3 years after planting a tree diversity experiment, soil microbial community diversity (but not AM fungal diversity) tracked the experimentally varied composition of tree communities.

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ABSTRACT

While the relationship between plant and microbial diversity has been well studied in grasslands, less is known about similar relationships in forests, especially for obligately symbiotic arbuscular mycorrhizal (AM) fungi. To assess the effect of varying tree diversity on microbial alpha- and beta-diversity, we sampled soil from plots in a high-density tree diversity experiment in Minnesota, USA, 3 years after establishment. About 3 of 12 tree species are AM hosts; the other 9 primarily associate with ectomycorrhizal fungi. We used phospho- and neutral lipid fatty acid analysis to characterize the biomass and functional identity of the whole soil bacterial and fungal community and high throughput sequencing to identify the species-level richness and composition of the AM fungal community. We found that plots of differing tree composition had different bacterial and fungal communities; plots with conifers, and especially *Juniperus virginiana*, had lower densities of several bacterial groups. In contrast, plots with a higher density or diversity of AM hosts showed no sign of greater AM fungal abundance or diversity. Our results indicate that early responses to plant diversity vary considerably across microbial groups, with AM fungal communities potentially requiring longer timescales to respond to changes in host tree diversity.

Keywords: biodiversity-ecosystem functioning; Gymnosperms; IDENT; *Juniperus virginiana*; PLFA; highthroughput sequencing

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INTRODUCTION

A growing number of studies indicate that soil microbes significantly influence the distribution of plants, determining which plants are present at a given site by allowing them to germinate and thrive (van der Heijden et al. 1998; Callaway et al. 2004; van der Heijden, Bardgett and van Straalen 2008). Conversely, other research suggests that microbes are largely ‘passengers’, becoming locally abundant in response to the presence of suitable plant hosts (Hart, Reader and Klironomos 2001; Prober et al. 2015; Schlatter et al. 2015; Leff et al. 2018). Habitat filtering may also shape the distribution of both microbes and plants (Zobel and Öpik 2014), such that patterns of co-occurrence in both groups emerge as a result of shared environmental preferences (Landis, Gargas and Givnish 2004; Klimek et al. 2015; Zhou et al. 2016). Disentangling the drivers of plant and microbial community richness and composition in natural surveys (e.g. de Vries et al. 2012) can be challenging due to the myriad of co-varying biotic and abiotic factors. As such, experiments in which plant diversity is controlled provide important opportunities to assess the extent to which plant diversity shapes microbial richness and composition in the field (Tilman, Isbell and Cowles 2014).

Considerable research using both observational surveys and manipulative experiments have documented a positive relationship between plant and microbial community richness (alpha-diversity) (Zak et al. 2003; Chen et al. 2017; Yang et al. 2017; Cline et al. 2018; Porazinska et al. 2018). At the same time, many other studies have found no evidence for the same relationship (Kivlin and Hawkes 2011; Li et al. 2015; Prober et al. 2015; Navrátilová et al. 2019). While the amount of covariation between the alpha-diversity of plants and microbes appears to be study-dependent, the relationship between plant and microbial community composition (beta-diversity) is more consistent. Significant associations between plant and microbial beta-diversity have been demonstrated in many different study systems: natural and designed diversity experiments in grasslands (Prober et al. 2015; Chen et al. 2017; Cline et al. 2018; Leff et al. 2018; Li et al. 2018), forests and plantations (Mueller et al. 2014; Barberán et al. 2015; Li et al. 2015; Nguyen et al. 2016; Pei et al. 2016; Yang et al. 2018), and peatlands (Bragazza et al. 2015). A number of studies have used phospholipid-derived fatty acid (PLFA) analysis to assess which microbial groups contribute most strongly to these plant-induced shifts in microbial community composition and have demonstrated that the observed community shifts are often linked to particular groups, such as Gram-positive or -negative bacteria, actinomycetes or different types of fungi (Hackl et al. 2005; Chung et al. 2007; Zechmeister-Boltenstern, Michel and Pfeffer 2011; Docherty et al. 2015; Chodak, Klimek and Niklińska 2016; Schmidt et al. 2017).

Because arbuscular mycorrhizal (AM) fungi are obligate plant symbionts, it is often assumed that AM fungal alpha- and beta-diversity patterns are closely linked to the richness and composition of their hosts at a variety of spatial scales (Treseder and Cross 2006; Tedersoo et al. 2012; Peay, Baraloto and Fine 2013). A number of studies have documented a positive relationship between plant and AM fungal alpha-diversity (Burrows and Pfleger 2002; Landis, Gargas and Givnish 2004; Chung et al. 2007; Peay, Baraloto and Fine 2013; Hiiesalu et al. 2014; Henning et al. 2018), but others have found no significant covariation (Lovelock and Ewel 2005; Antoninka, Reich and Johnson 2011; Lekberg et al. 2013). The absence of a positive relationship between plant and AM fungal alpha-diversity may be due to the fact that AM

fungi have low host specificity (Smith and Read 2008), so other factors such as rates of plant belowground C allocation (Adair et al. 2009) or favorable environmental conditions such as high soil moisture (Pei et al. 2016) may be more important in driving AM fungal richness. Like the patterns for bacterial and other fungal communities, however, the beta-diversity of AM fungi and plant communities has been found to be positively associated in many study systems (Burrows and Pfleger 2002; Lovelock and Ewel 2005; Antoninka, Reich and Johnson 2011; Yang et al. 2017). This latter trend is likely related to the fact that while AM fungi are rarely host specific, they do show significant growth preferences in and across hosts (van der Heijden et al. 1998; Bever 2002), which can result in significant specificity among AM fungal-host networks (Sepp et al. 2019).

Increasing availability of sequence data for both microbial taxa and plants has also made it feasible to assess whether the phylogenetic structure of plant communities significantly affects microbial community structure independent of changes in just species richness. Some studies have found that phylogenetic distance among plant biotrophs tends to increase with distance in host communities (Tedersoo et al. 2013; Liu et al. 2016), but others have not (Calatayud et al. 2016). To our knowledge, the only test of this relationship for mutualistic soil fungi involves the study of Nguyen et al. (2016), who showed that the alpha- and beta-diversity of ectomycorrhizal (EM) fungal communities were both significantly positively associated with host phylogenetic diversity. Interestingly, the authors were able to link the effect of plant phylogenetic diversity to fungal host specificity, such that plots with both gymnosperm and angiosperm tree hosts had higher EM fungal richness and greater compositional dissimilarity (relative to host monocultures) due to co-presence of host specialized taxa. The extent to which the same pattern applies for AM fungi, which have notable differences in host specificity compared with EM fungi, is unclear.

Although some general patterns regarding plant-microbial diversity linkages are beginning to emerge, studies of the relationship between plant and microbial richness and composition in forests (Li et al. 2015; Nguyen et al. 2016; Pei et al. 2016; Vitali et al. 2016; Noreika et al. 2019), particularly with regard to AM fungi, are scarcer than those conducted in grass-dominated systems. Because forests represent a dominant ecosystem type in terms of land coverage and biomass (Crowther et al. 2015), testing the generalities observed in grassland systems will help assess the robustness of emerging plant-microbial diversity relationships. We took advantage of a recently established biodiversity experiment in which the diversity of early-successional, temperate tree communities was experimentally manipulated. About 3 years after their planting, we sampled soil from 50 plots ranging in tree species richness from 1 to 12 species to assess how aboveground plant diversity influenced belowground microbial community. We quantified soil microbial community biomass and composition using neutral lipid fatty acid (NLFA) and PLFA analyses. To further examine how the species-level richness and composition of AM fungi responded to host tree diversity we also used high-throughput sequencing. We hypothesized as follows:

- (1) Microbial beta-diversity would show stronger statistical dependence on plant beta-diversity than microbial alpha-diversity on plant alpha-diversity.
- (2A) AM fungal abundance (using AM fungal-specific N/PLFAs) would be highest in plots with high AM host abundance.

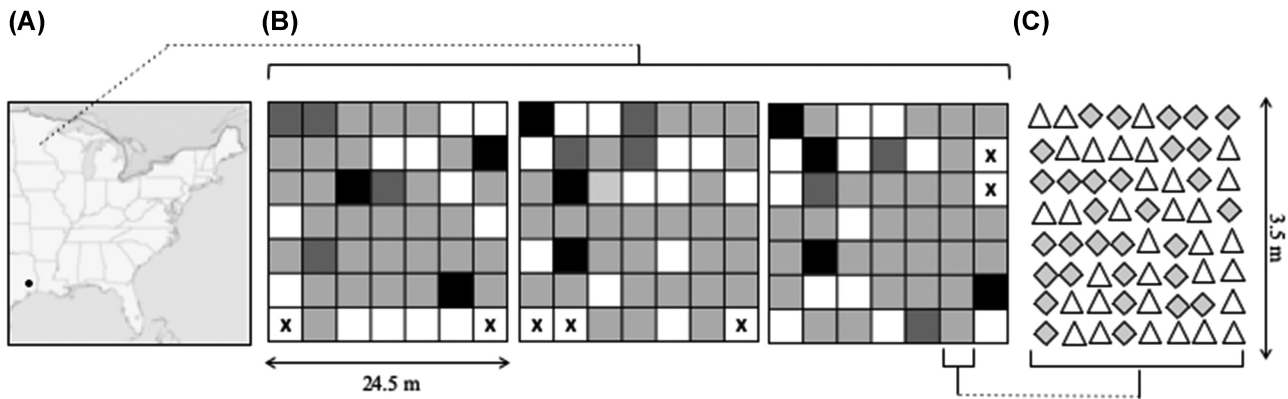


Figure 1. FAB experiment (A) is located at the Cedar Creek Ecosystem Science Reserve in East Bethel, Minnesota, USA. (B) It consists of 140 experimental plots (all those without an 'x') in three blocks. Each plot is planted with 1 (white), 2 (light grey), 5 (dark grey) or 12 (black) native tree species. (C) Each plot consists of 64 trees spaced at 0.5 m. Figure adapted from Grossman et al. (2019).

- (2B) AM fungal alpha- and beta-diversity would be highest in plots with high plant alpha- and beta-diversity, respectively.
- (2C) Host phylogenetic alpha-diversity would predict AM fungal alpha-diversity better than other dimensions of biodiversity, due to preferential growth relationships between specific AM fungal and host taxa.

METHODS

Study site: the forests and biodiversity experiment

This study was conducted in the high-density Forests and Biodiversity (FAB) tree diversity experiment (Fig. 1; Grossman et al. 2017), which was established in 2013 at the Cedar Creek Ecosystem Science Reserve in central Minnesota, USA (45°25'0" N, 93°10'0" W). This experiment, part of the International Diversity Experiment Network with Trees (IDENT; Tobner et al. 2014), consists of 8960 trees of 12 native, temperate species planted in a humid continental climate on an outwash plain with nitrogen-poor soils consisting of more than 90% sand. The site of the experiment was, prior to burning and tree planting, farmed (through 1967) and used as pasture (through 1977), then burned (in 1988 and 2013). At the time of the 2013 burning, it was an herbaceous old field consisting of grasses, forbs, and legumes; both AM and EM hosts were abundant. Tree species were chosen to span the seed plant phylogeny and included four gymnosperms, eastern red cedar (*Juniperus virginiana*) and white (*Pinus strobus*), red (*Pinus resinosa*), and jack (*Pinus banksiana*) pine; and eight angiosperms, red (*Quercus rubra*), pin (*Quercus ellipsoidalis*), white (*Quercus alba*), and bur (*Quercus macrocarpa*) oaks, red maple (*Acer rubrum*) and box elder (*Acer negundo*), paper birch (*Betula papyrifera*), and basswood (*Tilia americana*). Of these species, *J. virginiana*, *A. negundo* and *A. rubrum* are recognized as primarily AM hosts, while the mycorrhizal associations of the other nine species tend to be dominated by EM fungi (Brundrett, Murase and Kendrick 2008). When the FAB experiment was planted, all bare root seedlings were coated with a commercially available AM and EM inoculant powder (Bio Organics, New Hope, PA, USA) and mulched with non-sterile wood chips from non-native western red cedar (*Thuja plicata*), which likely also carried fungal inoculum.

Trees were planted 0.5 m apart on an orthogonal grid in 140 plots of 64 trees (9.25 m²). Plots are distributed randomly with respect to treatment in three blocks (600 m²) with no space between plots within the same block. Plots have a tree species

richness of 1, 2, 5 or 12 species, but in this study, we sampled only in a subset of plots containing 1, 2 or 12 species. Because our original motivation was to assess the relationship between experimentally imposed tree diversity and AM diversity and abundance, we excluded most plots with only EM hosts. Specifically, we sampled only from plots containing AM host species (*A. negundo*, *A. rubrum* or *J. virginiana*) and, as a control, from monocultures of two known EM hosts: one gymnosperm (*P. resinosa*) and one angiosperm (*B. papyrifera*). We excluded five-species polycultures because they were not replicated compositionally across the experiment. One- and two-species plots were triplicated across the experiment and 12-species plots were replicated 10 times. Given this, and following the loss of samples from 5 plots, our study focuses on soils from 50 plots of 16 distinct compositions: 5 monocultures (N = 15), 10 bicultures (N = 25; 5 biculture samples were lost), and the 12-species polyculture (N = 10). Actual sample sizes varied upon the analyses we performed and are given in Table 1.

Soil sampling

We collected soil samples twice: on 23–24 June and 24 August 2016. Recent rainfall prior to both events was similar, so the soil was moist, but not waterlogged when sampled. June soil samples were used to assess environmental variables (soil moisture, pH and phosphorus); August samples were collected for sequencing and lipid analysis. In both cases, surface vegetation and mulch were pushed aside and three 2.5 × 10 cm deep cores were taken at points in the interior of each plot (>1.0 m from plot edges). Cores were pooled and stored in plastic bags, and sampling equipment was sterilized with ethanol between plots to prevent cross-contamination. Samples were kept in a dark cooler and pooled at the plot level following collection.

Soil analysis

Soil moisture of all plots was measured in the field using a time-domain reflectometer. Samples collected on that date for environmental characterization were processed upon collection. One subset of fresh soil samples from all plots was oven-dried for 24 hours at 60°C and ashed at 550°C. Phosphorus content was measured using the sulfuric acid digestion with absorbic acid (APHA 1999). A second subset of fresh subsamples was diluted with DDI water (20 mL to 10 g soil) prior to pH measurements of resulting soil slurries.

Table 1. Plot-level tree community composition, soil analysis, lipid analysis and sequenced AMF richness by treatment. As appropriate, standard error is given in parentheses. Columns with bold titles and letter subscripts indicate significant differences by treatment via simple linear regression and post-hoc testing at $\alpha = 0.05$; columns without subscripts indicate no significant difference by treatment. Sample sizes for each analysis are given below plot composition for each treatment. All PLFA and NLFA values are given in terms of nmol g soil⁻¹. Soil moisture and phosphorus are presented as percentages. Fungal P/NLFA represent total mass for general markers 18:1 ω 9c and 18:2 ω 6,9c.

Plot composition (Nsoil / Nlipid / Nseq)	Tree richness	Host richness	Host density	Soil moisture	Soil pH	Soil phosphorus	Total		Fungal		Bacterial		Fungal		AM	
							PLFA	NLFA	PLFA	NLFA	PLFA	NLFA	PLFA	NLFA	PLFA	NLFA
<i>A. negundo</i> (3/3/3)	1	1	100%	4.67 (0.627)	6.81 (0.119)	0.053 (0.019)	9.37 (4.47)	10.2 _a (4.91)	0.704 (0.371)	1.24 _a (0.535)	1.00 (0.450)	2.39 (0.786)	0.412 _a (0.261)	2.65 (1.46)	8 (2)	8 (2)
<i>A. negundo</i> (3/3/3)	1	1	100%	6.11 (1.41)	6.56 (0.100)	0.083 (0.018)	8.76 (2.25)	8.96 _a (2.68)	0.904 (0.331)	1.20 _a (0.373)	2.00 (1.14)	3.09 (0.703)	0.445 _a (0.198)	1.16 (0.162)	5 (1)	5 (1)
<i>J. virginiana</i> (3/3/2)	1	1	100%	4.77 (1.86)	6.48 (0.076)	0.073 (0.050)	33.2 (28.0)	37.0 _{a,b} (5.27)	3.65 (3.18)	4.09 _{a,b} (1.71)	6.22 (2.73)	2.26 (1.50)	0.149 _a (0.149)	2.46 (1.70)	3 (0.4)	3 (0.4)
<i>A. negundo</i> - <i>A. rubrum</i> (2/2/1)	2	2	100%	7.93 (0.885)	6.86 (0.125)	0.045 (0.025)	48.1 (28.5)	26.4 _a (26.4)	3.86 (2.73)	2.14 _a (2.14)	5.50 (5.50)	3.22 (0.379)	6.65 _{a,b} (6.05)	0.737 (0.737)	10 (N = 1)	10 (N = 1)
<i>A. rubrum</i> - <i>J.</i> <i>virginiana</i> (2/2/2)	2	2	100%	7.04 (1.49)	6.39 (0.100)	0.135 (0.105)	19.5 (11.9)	8.17 _a (8.05)	1.26 (0.130)	0.578 _a (0.578)	0.481 (0.473)	2.54 (0.003)	0.437 _a (0.181)	2.34 (2.32)	10 (2)	10 (2)
<i>A. negundo</i> - <i>Q. alba</i> (3/3/3)	2	1	50%	5.53 (1.76)	6.65 (0.122)	0.010 (0.010)	17.4 (2.76)	20.3 _a (6.38)	1.46 (0.253)	2.05 _a (1.06)	3.03 (1.44)	4.19 (0.631)	0.791 _a (0.214)	5.06 (1.34)	5 (2)	5 (2)
<i>A. negundo</i> - <i>Q.</i> <i>ellipsoidalis</i> (3/2/3)	2	1	50%	3.70 (1.35)	6.81 (0.212)	0.023 (0.019)	9.47 (4.53)	4.56 _a (2.91)	0.983 (0.502)	0.506 _a (0.312)	0.401 (0.263)	2.18 (0.864)	0.400 _a (0.232)	1.38 (0.922)	7 (0.3)	7 (0.3)
<i>A. rubrum</i> - <i>P.</i> <i>banksiana</i> (3/2/3)	2	1	50%	5.58 (1.40)	6.48 (0.111)	0.097 (0.038)	6.91 (1.70)	18.0 _a (3.90)	0.498 (0.012)	1.44 _a (0.589)	2.97 (1.09)	1.22 (0.309)	0.301 _a (0.100)	0.938 (0.766)	8 (1)	8 (1)
<i>A. rubrum</i> - <i>T.</i> <i>americana</i> (3/2/3)	2	1	50%	3.68 (0.454)	6.75 (0.196)	0.040 (0.023)	9.56 (0.410)	9.41 _a (1.99)	1.01 (0.023)	0.904 _a (0.218)	0.654 (0.177)	2.76 (0.591)	0.424 _a (0.062)	2.61 (0.375)	9 (1)	9 (1)
<i>J. virginiana</i> - <i>P.</i> <i>banksiana</i> (2/2/2)	2	1	50%	6.71 (0.235)	6.62 (0.225)	<0.001	17.1 (11.8)	32.1 _{a,b} (21.2)	1.187 (0.522)	2.37 _a (1.57)	3.15- ⁺ 1.03 ₁₅ (1.09)	3.35 (2.24)	0.363 _a (0.153)	5.48 (3.51)	7 (1)	7 (1)
<i>J. virginiana</i> - <i>P.</i> <i>resinosa</i> (2/2/2)	2	1	50%	4.96 (1.82)	6.97 (0.205)	0.085 (0.035)	21.9 (4.94)	25.5 _a (3.19)	1.88 (1.42)	2.10 _a (0.522)	2.24 (0.530)	3.35 (2.24)	0.747 _a (0.480)	5.53 (1.41)	8 (0)	8 (0)
<i>J. virginiana</i> - <i>Q.</i> <i>rubrum</i> (2/1/2)	2	1	50%	6.48 (0.160)	6.74 (0.130)	0.170 (0.010)	32.9 (N = 1)	87.2 _b (N = 1)	2.28 (N = 1)	9.17 _b (N = 1)	7.93 (N = 1)	6.77 (N = 1)	1.14 _b (N = 1)	<0.00 (N = 1)	10 (6)	10 (6)
<i>J. virginiana</i> - <i>T.</i> <i>americana</i> (3/3/3)	2	1	50%	5.59 (1.86)	6.58 (0.263)	0.013 (0.013)	13.6 (4.22)	10.7 _a (5.09)	1.46 (0.764)	0.502 _a (0.469)	1.38 (0.764)	3.58 (1.43)	0.673 _a (0.298)	1.10 (0.983)	5 (0)	5 (0)
12 Species (10/10/10)	12	3	25%	5.60 (0.641)	6.60 (0.060)	0.034 (0.012)	14.9 (3.29)	17.5 _a (4.25)	1.14 (0.283)	1.54 _a (0.459)	2.13 (0.75)	3.63 (1.01)	0.518 _a (0.116)	2.51 (0.903)	6 (0.7)	6 (0.7)
<i>B. papyrifera</i> (3/3/1)	1	0	0%	6.38 (1.21)	6.95 (0.059)	0.057 (0.037)	14.9 (4.63)	8.38 _a (5.29)	1.28 (0.386)	1.16 _a (0.579)	1.48 (1.11)	4.39 (1.24)	0.636 _a (0.292)	1.15 (0.574)	10 (N = 1)	10 (N = 1)
<i>P. resinosa</i> (3/3/3)	1	0	0%	5.42 (1.03)	6.61 (0.039)	0.063 (0.009)	11.4 (5.31)	8.27 _a (2.52)	0.850 (0.463)	1.09 _a (0.386)	1.36 (0.519)	2.27 (0.518)	0.475 _a (0.330)	1.18 (0.472)	10 (3)	10 (3)

Microbial community lipid analysis

Samples designated for lipid analysis were originally stored at -20°C following collection, then thawed and freeze-dried. Though the original freeze and thaw step may have altered samples' lipid content, samples from all plots experienced the same treatment; we expect changes to be, therefore, independent with respect to the diversity of samples' plots of origin. Following freeze-drying, we extracted total soil lipids from a 10 g subsample from each plot and quantified both neutral lipid fatty acids (NLFA) and PLFA (Schmidt et al. 2017). Dissolved fatty acids were extracted from 2 g of freeze-dried soil through three extractions with a 1:1:0.9 chloroform to methanol to citrate buffer. Fatty acids were then converted to methyl esters through acid methylation and analyzed on a GC-MS (Agilent, HP DB5 column) spectrometer. Using an internal standard (13:0 tridecanoic methyl ester) for quantification, we converted peak areas to nmol g soil^{-1} . We quantified abundance of 24 microbial lipids (12:0, 13:0, 14:0, 15:0, i15:0, a15:0, 16:0, Me16:0, 16:1 ω 5c, 16:1 ω 7c, 16:1 ω 9c, 17:0, a17:0, i17:0, cy17:0, 18:0, Me1018:0, 18:1 ω 7c, 18:1 ω 9c, 18:1 ω 9t, 18:2 ω 6,9c, 19:0, cy19:0 and 20:0) and for each of these, calculated its mol %, or relative abundance, of a sample's total lipid mass. We considered a sample's total microbial biomass to be the sum of mass of all lipids present in the sample and also considered particular lipids as biomarkers of particular microbial groups (Wilkinson et al. 2002; Fierer, Schimel and Holden 2003; Balsler, Treseder and Ekenler 2005; McKinley, Peacock and White 2005). These include markers of Gram-positive (i15:0, a15:0, a17:0) and Gram-negative (16:1 ω 7c, 16:1 ω 9c, 18:1 ω 7c, 18:1 ω 9t) bacteria, actinomycetes (Me16:0, Me1018:0), anaerobic bacteria (cy19:0), general fungi (18:1 ω 9c, 18:2 ω 6,9c) and AM (16:1 ω 5c; including comparisons with NLFA per Ngosong, Gabriel and Ruess 2012).

AM fungal community analysis

The same soil samples collected in August 2016 for PFLA analysis were also used to assess AM fungal community richness and composition. We thawed subsamples from all plots and extracted DNA from 250 mg of each pooled soil sample using PowerPlant[®] Pro DNA isolation kit (MoBio Laboratories Inc. Solana Beach, CA). We then used the two-step PCR protocol of Lekberg et al. (2018) to generate amplicon pools for each sample. The first PCR step entailed amplification using the universal eukaryotic primer WANDA (SI of Dumbrell et al. [2011]) and an AM-fungal specific primer, AML2 (Lee, Lee and Young 2008). For each primer, we mixed 7 aliquots of each primer that also had varying numbers of Ns (1–7) to increased length heterogeneity in the amplicon pool. All first step PCR reactions were carried out in 12.5 μL reaction volumes containing 1 μL of DNA extract as template, 20 μmol of each primer in 1X GoTaq[®] Green Master Mix [(Green GoTaq[®] Reaction Buffer, 200 μM dATP, 200 μM dGTP, 200 μM dCTP, 200 μM dTTP and 1.5 mM MgCl_2) Promega, USA]. Thermocycling conditions were as follows: initial denaturation at 95°C for 2 min followed by 29 cycles at 95°C for 1 min, 54°C for 1 min and 72°C for 1 min, with a final elongation for 10 min at 72°C . All reactions were analyzed by 1.5% agarose gel electrophoresis using a 100 bp ladder to confirm the presence of target amplicons. The second PCR step added was used to add on unique forward and reverse GOLAY barcodes and Illumina flow-cell adaptors (P5 and P7, Illumina Inc., San Diego, CA, USA) for each sample. PCR reagents was identical, although a 1:10 dilution of first step PCR was used as DNA template. Thermocycling

conditions were as follows: initial denaturation at 95°C for 1 min followed by 10 cycles at 95°C for 30 seconds, 60°C for 30 sec and 68°C for 1 min, with a final elongation for 5 min at 68°C . Step 2 PCR products were purified and normalized using a 'Just-a-Plate'[®] kit (Charm Biotech, San Diego, CA, USA) and pooled to equimolar concentrations, and then sequenced at the University of Idaho's Institute for Bioinformatics and Evolutionary Studies (iBEST) genomics resources core (<http://www.ibest.uidaho.edu/>; Moscow, ID) on an Illumina MiSeq sequencing platform (Illumina Inc., San Diego, CA) using v2 (2×250 bp) chemistry.

The samples, while uniquely barcoded, were mixed with additional samples on a shared MiSeq run, resulting in a total of only 94 513 sequence reads. Forward only Illumina sequence files were first demultiplexed and then denoised and dereplicated using DADA2 (Callahan et al. 2016) and trimmed reads to 200 bp, resulting in 66 573 sequences remaining following quality filtering. Sequence variants (SVs) were then close-reference clustered using the MaarjAM database (Öpik et al. 2010), removing all sequences that did not match at least 80% identity and 80% coverage to sequences within MaarjAM. This resulted in the removal an additional 42 245 sequences (likely due our use of a relatively low annealing temperature, which facilitated non-specific amplification). Taxonomy was assigned using the BLAST function in QIIME2 and a 97% sequence similarity threshold, resulting in assignments to 23 873 sequence reads that were at least 97% identical over 90% of their sequence to those in MaarjAM (all reads lower than this criteria, which represented ~ 400 sequences, were removed from the final dataset). Any sequence reads present in negative controls were subtracted from sample read counts following Nguyen et al. (2015). Raw .fastq files of samples as well as negative controls were uploaded to the NCBI Sequence Read Archive under BioProject PRJNA560250.

Statistical analyses

All analyses were carried out using the R statistical computing platform v. 3.5.1 (R Core Team 2018).

The FAB plots varied in tree species richness, but also in phylogenetic diversity and functional diversity (Grossman et al. 2017). To assess which, if any, of these dimensions of tree diversity might best predict microbial diversity, we calculated plot-level tree phylogenetic and functional diversity in terms of mean pairwise distance (MPD; Webb et al. 2002) using the 'SIMPER' function in the 'picante' R package (Kembel et al. 2010). Phylogenetic mean pairwise distance (hereafter phylogenetic diversity) was computed using the (Zanne et al. 2014) phylogeny. Functional mean pairwise distance (hereafter functional diversity) represents the multidimensional Euclidean distance separating species-level values of six leaf traits that we believed might affect belowground diversity: specific leaf area and leaf water, nitrogen, phosphorus, lignin, and condensed tannin content. Species-level trait values (Supplementary Table S1, Supporting Information) were extracted from Grossman et al. (2019). All traits values were scaled and centered to a mean of 0 and a variance of 1 prior to calculation of functional diversity.

Following Schmidt et al. (2017), we used several functions (in italics) from the 'vegan' R package to assess microbial community diversity (Oksanen et al. 2008). We used non-metric multidimensional scaling (NMDS) to visualize variation in total NLFA/PLFA profiles (McCune and Grace 2002; *metaMDS*). Relative abundances of each lipid marker recovered per plot were used as raw input into the NMDS procedure and predictors of

microbial community composition were fit onto NMDS ordination using 'envfit'. We selected a group of candidate predictors based on a *priori* expectations of what factors would shape microbial community composition. We fit only those predictors that both were strongly associated with each axis of ordination and that were not significantly correlated with each other (via Spearman's correlation test). PERMANOVA (adonis) was used to assess the dependence of PLFA-derived microbial community composition on plot composition. SIMPER analysis pinpointed which particular microbial groups contributed most to differences among plot types (SIMPER). Following (Nguyen et al. 2016), we also used a Mantel test ('mantel' in the 'Vegan' package) to assess covariance between the AM fungal and plant communities in sampled plots.

To estimate the phylogenetic diversity of AM fungal communities from sampled plots, we used a pre-aligned April 2015 version of the full MaarjAM database (provided by M. Opik, pers. comm.), and then constructed a phylogenetic tree of all available sequences using the 'phangorn' package in R (Schliep 2019). We created a distance matrix assuming equal base frequencies (JC69 in the 'dist.ml' function) and built a neighbor-joining tree (Supplementary Figure 1, Supporting Information) from this matrix using the 'NJ' function. We pruned this tree to include only taxa identified from our samples and then followed the same protocol described above (in reference to host plant communities) to calculate phylogenetic mean pairwise distance in 'picante.' We chose to weight AM fungal phylogenetic diversity based on relative abundance of reads per taxon.

Relationships between soil, plant community, and microbial community indices were further assessed using simple linear regression. As appropriate, indicators of microbial community diversity (lipid or sequence data) or abundance (lipid data) were regressed on an indicated predictor, giving an equation of the form

$$\text{Response} \sim \text{Predictor} * \beta + \varepsilon,$$

where β indicates the regression coefficient for the predictor and ε encapsulates model error. Normality and constancy of variance were assessed and outliers removed as necessary. Data were not transformed for linear modeling as they were generally normal and homoscedastic. In cases in which ANOVA indicated a significant relationship between the response and predictor, *post-hoc* testing was carried out using the 'HSD.test' in the 'agricolae' package (de Mendiburu 2016).

RESULTS

Tree community diversity and soil microbial community diversity are linked.

Generally, soil qualities, lipid content and microbial diversity did not differ consistently among treatments (Table 1, PERMANOVA of all phospholipids: $F = 0.930$, $r^2 = 0.316$, $P = 0.58$). However, some facets of tree diversity and soil conditions, did significantly influence microbial community structure (Fig. 2). In particular, the proportion of AM hosts (*Acer* spp. and *J. virginiana*) in the plot ($r^2 = 0.286$, $P = 0.010$) as well as plot soil phosphorus ($r^2 = 0.203$, $P = 0.047$) had a high level of fit to the phospholipid ordination along NMDS axis 2. Multidimensional functional diversity of the plant community's leaf traits ($r^2 = 0.173$, $P = 0.064$) also seemed to be associated with this ordination axis to a lesser extent. In

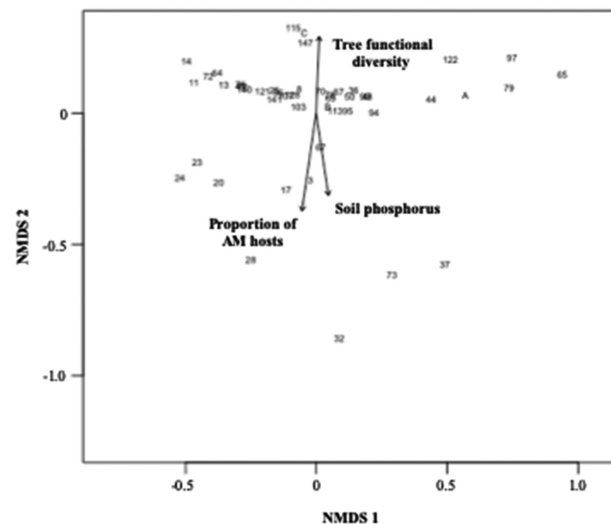


Figure 2. NMDS of PLFA data indicates that plots (shown as arbitrarily numbered points) differ in their microbial community composition. Tree functional diversity, the proportion of AM hosts in a plot, and soil phosphorus all show some degree of fit to the ordination in parallel with the second NMDS axis. However, none of the soil or tree community metrics we calculated explain variation in community microbial community diversity captured in NMDS axis 1.

contrast, none of the soil or plant community metrics aligned with spread in NMDS axis 1.

The primary lipids driving separation across the NMDS axes were those associated with total microbial biomass (12:0, 13:0, 19:0, 20:0) (Supplementary Figure 2, Supporting Information). However, certain microbial groups also appeared to have differential responses to changes in tree diversity. Across all plot compositions, the 18:1 ω 9t lipid, indicating the presence of Gram-negative bacteria, was the primary contributor to community dissimilarity, causing 3.4% to 8.8% dissimilarity between plots (Table 2; Supplementary Table S2, Supporting Information). The cy19:0 lipid, indicating anaerobic bacteria, and the i15:0 lipid, indicating Gram-positive bacteria, were generally the next most important contributors to plot dissimilarity. Though fungal-derived lipids were often minor contributors to plot differences in microbial composition, they were generally less important than those of bacteria. No significant trends were also observed in total microbial neutral to phospholipid ratios or in abundances of fungal neutral or phospholipids (results not shown).

Across comparisons among all five monocultures sampled and between each monoculture and the 12-species polyculture, plots containing *J. virginiana* were most compositionally dissimilar to other plots (via SIMPER analysis; Table 3). Monocultures of *J. virginiana* were most compositionally similar to monocultures of fellow gymnosperm *P. resinosa* (dissimilarity = 0.373), and had dissimilarity scores between 0.40 and 0.46 for other monocultures and for 12-species plots, which contained 7–8% *J. virginiana* individuals.

In bivariate models assessing the effect of various dimensions of tree community diversity on particular metrics of microbial community composition, plots with more angiosperms had higher concentrations of Gram-positive ($t = 2.77$, $P = 0.008$, $r^2 = 0.129$) and Gram-negative ($t = 1.70$, $P = 0.097$, $r^2 = 0.041$) bacteria and actinobacteria ($t = 2.31$, $P = 0.025$, $r^2 = 0.088$). The ratio of Gram-negative to Gram-positive bacteria was also lower in plots with more angiosperms ($t = -2.11$, $P = 0.041$, $r^2 = 0.071$). This was especially true in plots with higher densities of *J. virginiana*.

Table 2. SIMPER analysis of PLFA data for monocultural plots. For each pair of monocultures, overall Bray-Curtis dissimilarity is given (larger values indicate greater dissimilarity). All fatty acids contribution to 1% or greater of dissimilarity between monocultures are identified along with the class of microbe for which they are an indicator and their absolute contribution to mean fatty acid content in surveyed plots. Comparisons between monocultures and 12-species polycultures are given in Supplementary Table S2 (Supporting Information).

Overall															
Bray-Curtis dissimilarity	Fatty acids	Indicator status	Contribution to dissimilarity		Average fatty acid abundance (%)		Bray-Curtis dissimilarity	Fatty acids	Indicator status	Contribution to dissimilarity		Average fatty acid abundance (%)			
			A. negundo	A. rubrum	A. negundo	A. rubrum				A. rubrum and P. resinosa monocultures	A. rubrum	A. rubrum	A. rubrum	P. resinosa	
A. negundo and A. rubrum monocultures	0.189	18:1 ω 9t	Gram-negative bacteria	0.053	5.49	10.9	A. rubrum and P. resinosa monocultures	0.210	18:1 ω 9t	Gram-negative bacteria	0.034	10.9	A. rubrum	8.00	
		19:0 cyclo	Anaerobic	0.018	5.28	5.70			15:0 iso	Gram-positive bacteria	0.023	5.64		3.52	
		15:0 iso	Gram-positive bacteria	0.016	5.13	5.64			16:1 ω 5c	AM Fungi	0.021	4.61		3.31	
		18:2 ω 6,9c	S fungi	0.015	2.38	3.87			19:0 cyclo	Anaerobic	0.019	5.70		3.93	
		16:1 ω 5c	AM Fungi	0.015	3.76	4.61			16:0 10me	Actinomycetes	0.019	4.37		2.66	
		15:0 anteiso	Gram-positive bacteria	0.013	3.44	3.09			18:1 ω 9c	S fungi	0.017	5.80		4.11	
		16:0 10me	Actinomycetes	0.012	3.91	4.37			16:1 ω 7c	Gram-negative bacteria	0.015	4.21		2.84	
A. negundo and J. virginiana monocultures	0.451	18:1 ω 9t	Gram-negative bacteria	0.061	5.49	3.40	A. rubrum and B. papuyrifera monocultures	0.192	18:1 ω 9t	Gram-negative bacteria	0.044	10.9	A. rubrum	10.6	
		19:0 cyclo	Anaerobic	0.060	5.28	1.16			19:0 cyclo	Anaerobic	0.029	5.70		2.55	
		15:0 iso	Gram-positive bacteria	0.055	5.13	1.29			15:0 iso	Gram-positive bacteria	0.015	5.64		4.59	
		16:0 10me	Actinomycetes	0.041	3.91	1.05			16:1 ω 5c	AM Fungi	0.015	4.61		3.86	
		15:0 anteiso	Gram-positive bacteria	0.041	3.44	3.44			18:1 ω 9c	S fungi	0.014	5.80		5.01	
		16:1 ω 5c	AM Fungi	0.038	3.76	2.10			16:1 ω 7c	Gram-negative bacteria	0.013	4.21		3.59	
		18:1 ω 9c	S fungi	0.026	4.71	4.71			18:2 ω 6,9c	S fungi	0.012	3.87		3.60	
A. rubrum and J. virginiana monocultures	0.448	18:1 ω 9t	Gram-negative bacteria	0.088	10.9	2.67	J. virginiana and P. resinosa monocultures	0.373	18:1 ω 9t	Gram-negative bacteria	0.065	3.40	J. virginiana	8.00	
		19:0 cyclo	Anaerobic	0.058	5.70	0.775			19:0 cyclo	Anaerobic	0.046	1.16		3.93	
		15:0 iso	Gram-positive bacteria	0.055	4.37	0.702			16:1 ω 5c	AM Fungi	0.042	2.10		3.31	
		16:0 10me	Actinomycetes	0.043	5.64	2.02			18:1 ω 9c	S fungi	0.039	6.43		4.11	
		16:1 ω 5c	AM Fungi	0.039	4.61	1.74			15:0 iso	Gram-positive bacteria	0.036	1.29		3.52	
									15:0 anteiso	Gram-positive bacteria	0.027	0.629		2.53	
									16:0 10me	Actinomycetes	0.027	1.05		2.66	

Table 2. Continued

	Overall											
	Bray-Curtis dissimilarity	Fatty acids	Indicator status	Contribution to dissimilarity	Average fatty acid abundance (%)	<i>B. papyrifera</i> and <i>J. virginiana</i> monocultures	Bray-Curtis dissimilarity	Fatty acids	Indicator status	Contribution to dissimilarity	Average fatty acid abundance (%)	
<i>A. negundo</i> and <i>B. papyrifera</i> monocultures	0.235	18:1 ω 9t	Gram-negative bacteria	0.065	A. negundo 5.49	<i>B. papyrifera</i> and <i>J. virginiana</i> monocultures	0.408	18:1 ω 9t	Gram-negative bacteria	0.087	<i>B. papyrifera</i> 10.6	
		19:0 cyclo	Anaerobic	0.031	5.28			15:0 iso	Gram-positive bacteria	0.046	4.59	
		15:0 iso	Gram-positive bacteria	0.021	5.13			16:1 ω 5c	AM Fungi	0.038	3.86	
		15:0 anteiso	Gram-positive bacteria	0.017	3.44			16:0 10me	Actinomycetes	0.035	3.52	
		16:0 10me	Actinomycetes	0.015	3.91			19:0 cyclo	Anaerobic	0.033	2.55	
		16:1 ω 7c	Gram-negative bacteria	0.013	3.69			18:1 ω 9c	S fungi	0.028	5.01	
		18:2 ω 6,9c	S fungi	0.013	2.38					0.028	2.65	
					A. negundo 5.49						<i>B. papyrifera</i> 10.6	
												<i>J. virginiana</i> 3.40
												1.29
											2.10	
											1.05	
											1.16	
											6.43	
											0.629	
<i>A. negundo</i> and <i>P. resinosa</i> monocultures	0.220	18:1 ω 9t	Gram-negative bacteria	0.044	A. negundo 5.49	<i>B. papyrifera</i> and <i>P. resinosa</i> monocultures	0.205	18:1 ω 9t	Gram-negative bacteria	0.049	<i>B. papyrifera</i> 10.6	
		15:0 iso	Gram-positive bacteria	0.027	5.13			19:0 cyclo	Anaerobic	0.022	2.55	
		19:0 cyclo	Anaerobic	0.022	5.28			15:0 iso	Gram-positive bacteria	0.017	4.59	
		15:0 anteiso	Gram-positive bacteria	0.019	3.44			16:1 ω 5c	AM Fungi	0.017	3.86	
		16:0 10me	Actinomycetes	0.018	3.91			17:0 anteiso	Gram-positive bacteria	0.013	1.85	
		16:1 ω 5c	AM Fungi	0.017	3.76			16:0 10me	Actinomycetes	0.013	3.52	
		18:1 ω 9c	S fungi	0.010	4.71			18:1 ω 9c	S fungi	0.012	5.01	
								16:1 ω 7c	Gram-negative bacteria	0.012	3.59	
												1.10
												2.66
											4.11	
											2.84	

Table 3. Bray–Curtis dissimilarity of PLFA data associated with microbial communities in monoculture plots and 12-species polycultures in the FAB experiment. Dissimilarity ranges from 0 to 1, with higher values indicating greater difference in community composition. Bold monocultures are those of known AMF hosts. The 12-species polycultures contain each species noted here and 7 other non-AMF hosts. Monocultures of *J. virginiana* differ most from other plots.

	<i>A. negundo</i>	<i>A. rubrum</i>	<i>P. resinosa</i>	<i>B. papyrifera</i>	12-species
<i>A. negundo</i>					
<i>A. rubrum</i>	0.189				
<i>P. resinosa</i>	0.220	0.210			
<i>B. papyrifera</i>	0.235	0.192	0.205		
12-species	0.287	0.252	0.276	0.287	
<i>J. virginiana</i>	0.451	0.448	0.373	0.408	0.459

Plots with higher proportions of that species had lower concentrations of Gram-positive ($t = -2.99$, $P = 0.005$, $r^2 = 0.150$) and Gram-negative ($t = -2.02$, $P = 0.050$, $r^2 = 0.064$) bacteria and actinobacteria ($t = -2.70$, $P = 0.010$, $r^2 = 0.122$), and a higher ratio of Gram-negative to Gram-positive bacteria ($t = 2.86$, $P = 0.007$, $r^2 = 0.137$). Bivariate models also indicate that actinobacteria were marginally more abundant in plots with higher functional diversity in leaf traits ($t = -1.78$, $P = 0.086$, $r^2 = 0.067$).

2A. Neither host abundance nor soil characteristics shaped AM fungal abundance

Neither the absolute amount nor the relative abundance of phospholipids (Fig. 3) or neutral lipids corresponding to the AM fungal-specific 16:1 ω 5c were associated with plot composition. Indeed, no significant relationship between plot-level proportion of *A. negundo*, *A. rubrum*, both *Acer* spp., *J. virginiana* or all three AM hosts and absolute amounts or relative abundance of either AM fungal-specific phospholipids or neutral lipids (or the spore:hyphae ratio) in sampled soil was observed (Table 1). Additionally, in bivariate regression, soil moisture did not predict the species richness (or other diversity metrics) of AM fungi ($t = 0.793$, $P = 0.432$). Similarly, soil moisture also did not predict the concentration of the 16:1 ω 5c phospholipid marker ($t = 0.627$, $P = 0.534$).

2B and 2C. Tree diversity, regardless of dimension, had no effect on AM alpha-diversity and a weak effect on AM fungal community composition

AM fungal alpha-diversity was not significantly associated with AM host taxonomic (Fig. 4) or phylogenetic diversity (not shown). In contrast, AM fungal beta-diversity was significantly linked to AM host beta-diversity, albeit somewhat modestly (Mantel Test: $r = 0.087$, $P = 0.038$). The SIMPER analysis of the AM-fungal specific phospholipid 16:1 ω 5c also indicated that the presence and abundance of AM fungal hyphae contributed to differences microbial community composition among plots with different tree community composition (Table 2; Supplementary Table S2, Supporting Information). Plots with more angiosperms overall had a lower ratio of neutral to phospholipids associated with AM fungi ($t = -1.76$, $P = 0.086$, $r^2 = 0.045$), and the reverse was true in plots with higher proportions of *J. virginiana* ($t = 3.46$, $P = 0.001$, $r^2 = 0.200$).

Of 47 AM fungal taxa identified across the study, 24 were only found in plots with AM hosts (Supplementary Table S3, Supporting Information). The other 23 were found in control monocultures with only EM hosts among the woody vegetation. The read abundances of the 24 taxa present only in plots planted with AM

hosts was low (15 ± 3 reads total/plot, mean \pm s.e.). Read abundances did not differ significantly by AM host density ($F = 1.801$, $P = 0.172$; 25% AM host = 9 ± 3 , 50% AM host = 22 ± 7 , 100% AM host 14 ± 5 reads total/plot, mean \pm s.e.) and there was no clear AM fungal fidelity for particular AM host species (Supplementary Table S3, Supporting Information).

DISCUSSION

Overall, we found measurable but generally weak effects of tree community diversity on the whole soil microbial community, with the strongest relationships emerging between gymnosperm abundance and identity and bacterial abundance and identity. Our results also indicate that AM fungal communities appear either not to have responded to planted tree community diversity or to be in the very initial stages of response 3 years after experimental establishment.

Soil microbes have begun to respond to tree diversity, and especially that of gymnosperms

Our PLFA analyses suggest that the soil microbial community in this relatively young tree diversity experiment had begun to respond to plot-level differences in tree community composition. In contrast to past studies in controlled field experiments (e.g. Schmidt et al. 2017), soil microbial community diversity did not vary consistently with plant community diversity (Table 1). Yet particular dimensions of tree diversity did predict composition of the microbial community. Specifically, we found a high to moderate level of fit between the second NMDS axis of microbial diversity and two dimensions of plant diversity: the proportion of AM-associated hosts (*A. negundo*, *A. rubrum* and *J. virginiana*) in a plot and, following Cline et al. (2018), plant functional diversity in the plot (Fig. 2). Furthermore, despite the general condition of nitrogen, rather than phosphorus, limitation at the experimental site (Cedar Creek; Tilman 1984), microbial community composition also appeared to respond to soil phosphorus in this study system, corroborating past results (Kuramae et al. 2012; Chodak, Klimek and Niklińska 2016), including observations in the same study system (Johnson 1993).

In taxa-specific analyses, we found evidence that several bacterial groups may be especially responsive to variation in local abundance and diversity of gymnosperms, and of *J. virginiana* in particular (Williams et al. 2013). Sampled plots ranged from 0 to 100% in gymnosperm abundance and included mixtures of three pines (*P. banksiana*, *P. resinosa* and *P. strobus*) and *J. virginiana*. Lipid analysis indicates that Gram-positive and Gram-negative bacteria as well as actinomycetes became less abundant in plots with higher abundance of these gymnosperm species. This was especially true in plots enriched with *J. virginiana*, which seemed to

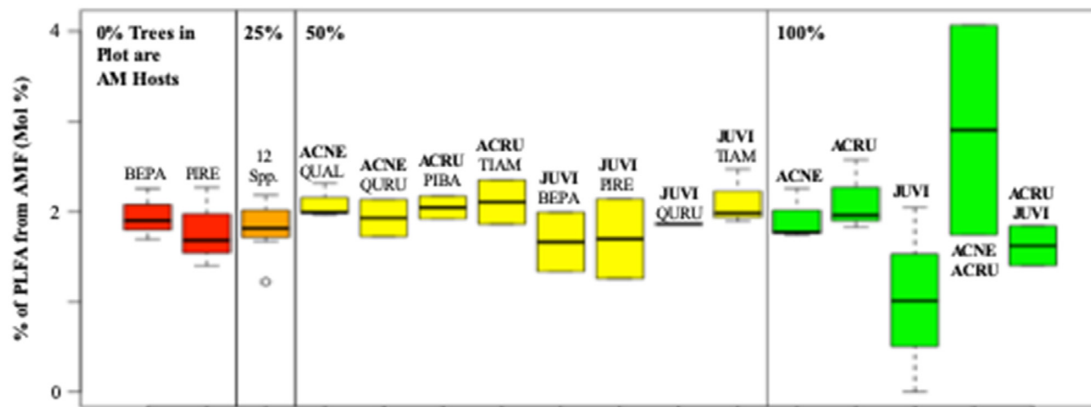


Figure 3. The abundance of AM fungal biomass (mol % of lipid 16:1 ω 5c) did not vary consistently with the proportion of AM hosts (*Acer negundo*/ACNE, *A. rubrum*/ACRU and *J. virginiana*/JUVI) in a given plot. Acronyms of AM hosts are in bold. Acronyms of non-AM hosts are as follows: *B. papyrifera* (BEPA), *P. banksiana* (PIBA), *P. resinosa* (PIRE), *Quercus alba* (QUAL), *Q. rubra* (QURU) and *T. americana* (TIAM).

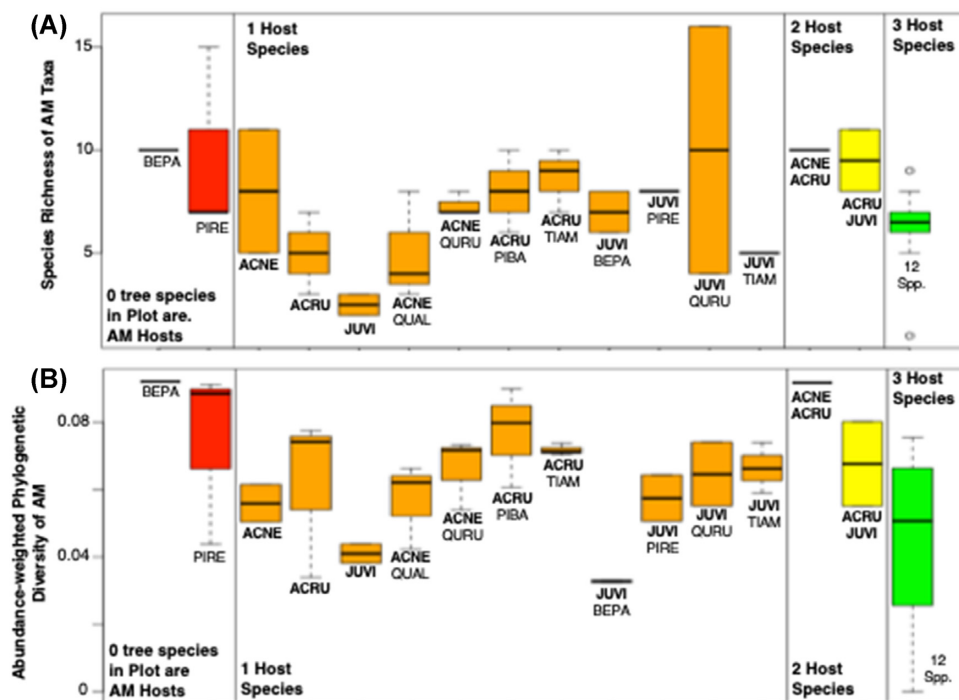


Figure 4. Neither the (A) richness of AM fungal OTUs nor the (B) abundance-weighted phylogenetic diversity of these AM taxa varied consistently with the richness of AM host species (*Acer negundo*, *A. rubrum* and *J. virginiana*). Species are labeled as in Fig. 3; acronyms of AM hosts are in bold.

function as an extreme gymnosperm, reducing the abundance of all three bacterial groups and especially depressing populations of Gram-positive bacteria relative to Gram-negative bacteria. Our SIMPER analyses reinforced this finding: monocultures of *J. virginiana* had substantially different microbial communities from other plots (Tables 2 and 3; Supplementary Table S2, Supporting Information). In general, these findings agree with those from other tree-dominated systems, in which lipid profiles of the bacterial community, in particular, have been shown to differ between angiosperm- and gymnosperm-dominated sites (Hackl et al. 2005; Zechmeister-Boltenstern, Michel and Pfeffer 2011; Chodak, Klimek and Nikińska 2016). Bacterial abundances seem to be particularly low under pine-dominated forest cover, perhaps due to the antimicrobial effects of pine secondary compounds (Hong et al. 2004). And tissues from *Juniperus* species in particular are known to possess antimicrobial

properties (Clark, McChesney and Adams 1990), even exceeding the antibacterial potency of streptomycin (Elliott, Elliott and Wyman 1993, but see Williams et al. 2013). Importantly, our finding of the particular effect of *J. virginiana* dominance suggests that particular gymnosperm taxa may significantly vary in the strength of their effects on soil microbial communities in tree diversity experiments.

AM abundance and diversity has not responded to host diversity or abundance

We found little evidence that experimentally manipulated tree community diversity in our experiment had altered either the abundance or diversity of the AM fungal community (Figs 3 and 4; Table 1). In contrast to past work on the relationship

between AM abundance and local host abundance (Lekberg et al. 2013) or biomass (Pei et al. 2016), there was no clear relationship between the density of AM hosts in a given plot and the abundance of AM fungal-derived lipids, in absolute or relative terms. Though plots with high proportions of *Acer* species had non-significantly higher levels of these lipids than other plots on average, *J. virginiana* abundance was not associated with an increase in AM fungi (Williams et al. 2013). Furthermore, the SIMPER analyses indicated that AM-associated lipids were more abundant in monocultures of *Acer* spp. and of non-AM hosts (*B. papyrifera* and *P. resinosa*) than in *J. virginiana* monocultures (Table 2; Supplementary Table S2, Supporting Information). This lack of dependence of AM abundance on host abundance held for both AM-specific 16:1 ω 5c neutral lipids as well as for 16:1 ω 5c phospholipids, which, while diagnostic of AM fungi, can also be produced by Gram-positive bacteria (Ngosong, Gabriel and Ruess 2012). Interestingly, these results suggest that despite being an AM host, *J. virginiana* has broadly suppressive effects on both AM fungal and bacterial abundances in soils beneath its canopy.

In contrast to most prior work on AM fungi in grasslands (Burrrows and Pfleger 2002; Landis, Gargas and Givnish 2004; Hiiesalu et al. 2014; Henning et al. 2018) and past findings focusing on EM fungi (Nguyen et al. 2016), we found no evidence that tree communities with either more AM hosts or more diverse AM tree hosts had a more diverse AM fungal community (Fig. 3). Indeed, in parallel to findings of Antoninka, Reich and Johnson (2011) in a grassland located directly adjacent to our experiment, we found qualitative patterns of higher AM fungal diversity in monoculture plots, including in monocultures of AM non-hosts. Inspection of the distribution of the 47 AM fungal taxa we identified through sequencing indicates that AM fungal diversity in plots with suitable hosts was generally low, with very few taxa appearing only in plots with AM hosts. Many of these taxa were also present in soil samples taken from sites close to the experiment but devoid of aboveground evidence of AM hosts (e.g. a bare stretch of sandy soil next to the experiment), but absent in negative controls. Taken together, this suggests our results reflect a lack of biological signal, rather than a methodological constraint. The recent evidence that plants' capacities to shape AM fungal communities may be confined to spatial scales (~30 cm, Rasmussen et al. 2018) an order of magnitude smaller than our plots (9.25 m²) may help explain the absence of any strong relationships between AM fungal and plant diversity. Furthermore, methodological constraints may have limited our capacity to fully document the AM fungal taxa present in surveyed communities. Low-volume (250 mg) soil samples such as those we collected can produce low read counts (Davison et al. 2012), which combined with the notable non-specific amplification we encountered, may have obscured our ability to discern clearer diversity patterns.

CONCLUSIONS

The relatively limited overall effects of plant community diversity on microbial community diversity were perhaps to have been expected given that only 3 years had passed between establishment of the FAB experiment and our soil sampling. Legacy effects from the site's pre-experimental vegetation, a post-agricultural, disturbed grassland, may, to this point, have a stronger impact on local microbial community composition and previous work in revegetating ecosystems indicates that microbial community development may lag years or decades behind plant community development (Oehl et al. 2011; Zhang et al. 2017). Whereas Li et al. (2015) showed evidence that microbial

communities can take several years to begin tracking plant communities, Porazinska et al. (2018) point out that the complex responses of microbes to the built-up of organic matter over longer time periods may ultimately override the influence of plant community composition. As such, we suggest that resampling of the same soil microbial community 5 to 10 years in the future will provide an excellent opportunity to assess the dependence of this relationship on temporal scale in early successional ecosystems (Piotrowski and Rillig 2008; Krüger et al. 2017).

DATA ACCESSIBILITY

All data are archived and publicly available at the LTER Network Data Portal: <https://portal.lternet.edu/nis/mapbrowse?packageid=knb-lter-cdr.683.1>.

SUPPLEMENTARY DATA

Supplementary data are available at [FEMSEC](https://www.femsec.org/) online.

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AUTHOR CONTRIBUTIONS

J.C.B., S.E.H. and P.B.R. designed the FAB experiment. A.J.B., J.J.G. and P.G.K. conceived and designed the study presented in this report. A.J.B. and J.J.G. conducted soil sampling and soil physical and chemical analysis. J.J.G. and J.G. performed N/PLFA and analyzed lipid data. J.J.G. and P.G.K. performed AM fungal sequencing and analyzed molecular data. All authors contributed to writing or editing the manuscript; J.J.G. and P.G.K. wrote the first draft.

Conflict of interest. None declared.

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