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LETTER

Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest

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Abstract

The complexities of the relationships between plant and soil microbial communities remain unresolved. We determined the associations between plant aboveground and belowground (root) distributions and the communities of soil fungi and bacteria found across a diverse tropical forest plot. Soil microbial community composition was correlated with the taxonomic and phylogenetic structure of the aboveground plant assemblages even after controlling for differences in soil characteristics, but these relationships were stronger for fungi than for bacteria. In contrast to expectations, the species composition of roots in our soil core samples was a poor predictor of microbial community composition perhaps due to the patchy, ephemeral, and highly overlapping nature of fine root distributions. Our ability to predict soil microbial composition was not improved by incorporating information on plant functional traits suggesting that the most commonly measured plant traits are not particularly useful for predicting the plot-level variability in belowground microbial communities.

Keywords

Bacteria, Barro Colorado Island, functional traits, fungi, microbial ecology, phylogeny, roots, soil, trees, tropical forest.

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INTRODUCTION

Soil microbes engage in complex feedbacks with plants (Wardle *et al*. 2004; Bever *et al*. 2013), although the specific mechanisms that link aboveground and belowground communities often remain unresolved. Predicted associations between plant and microbial distributions arise from known plant effects on soil conditions, including the amounts and types of organic carbon (C) inputs, soil pH, or soil nutrient availability through litter and root exudates (van der Heijden *et al*. 2008; Prescott & Grayston 2013). These soil modifications can have direct and indirect effects on local microbial communities by favouring the growth of symbiotic bacteria and fungi (including nitrogen-fixing bacteria and mycorrhizal fungi) or microbial pathogens (Wardle *et al*. 2004). However, plant and microbial assembly can also occur independently in response to similar abiotic properties of soil (Hines *et al*. 2006), or can occur at different spatial and temporal scales (Bardgett *et al*. 2005), making it difficult to identify generalisable patterns and associations between plants and belowground microbes.

Given the myriad of interactions between plants and microbes and their well-known impacts on ecosystem function (van der Heijden *et al*. 2008), it is often assumed that the composition of belowground microbial communities and aboveground plant communities will reflect one another. However, evidence to support this assertion is scarce (Prescott & Grayston 2013), with some studies showing that plant community composition is a significant predictor of overall bacterial and/or fungal community composition at regional or continental-scale studies (Prober *et al*. 2015), and other studies unable to find the same relationship (Talbot *et al*. 2014). Even when studies are tightly controlled, a correlation between plant species identity and overall bacterial or fungal community composition might (Jiang *et al*. 2012) or might not (McGuire *et al*. 2012) be present.

Various methodological and ecological factors could obscure the detection of tight associations between plants and belowground microbial communities. First, methodological constraints in microbial detection and quantification may make it difficult to identify patterns between above and belowground

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communities (Fierer & Lennon 2011). Second, if the environmental factors structuring plant communities are distinct from those that structure the overall composition of belowground microbial communities, relationships between individual plant species and microbial community structure would be unlikely. For example, some studies show that soil pH can have a large influence on the composition of soil bacterial communities, but little influence on plant species composition, contributing to an apparent disconnect between plant communities and the bacterial communities found in soil (Prober *et al*. 2015). Third, plants may influence belowground microbial communities via specific plant traits or attributes that are not predictable from plant species identity alone such as litter C : N ratio, root exudate production, or litter carbon chemistry. There is a growing body of evidence suggesting that functional traits or the evolutionary relatedness of plants can often be more useful predictors of plant effects on belowground biota than species identity (De Deyn & Van der Putten 2005). Finally, soil microbial communities may not respond to aboveground plant distributions, but rather to the fine-scale distributions of plant root networks, because the spatial scale of aboveground ground canopies and belowground distributions of plant species may not be equivalent (Jones *et al*. 2011).

In order to disentangle the associations between plant and belowground microbial communities, we combined detailed microbial community analyses with integrated assessments of belowground and aboveground plant distributions that considered the taxonomic identities of plants, the phylogenetic relationships among plants, and plant functional traits. To accomplish these objectives, we used marker gene sequencing to identify plant roots and characterise the bacterial and fungal communities found in each of 625 soil samples collected from a 50-ha tropical forest plot located on Barro Colorado Island, Panama, a plot that has been intensively studied by plant ecologists for decades and has been a focal point for tropical research investigating tree coexistence and the maintenance of plant diversity (Hubbell & Foster 1983). We used this data set to determine the extent to which the variability in the composition of soil bacterial and fungal communities across the plot was predicted by above and belowground plant species distributions. We tested the prediction that the association between plants and belowground microbial communities would be stronger for belowground than aboveground plant distributions because roots are expected to have important influences on the development of soil microbial communities. In addition, we tested the hypothesis that phylogenetic relatedness and the functional traits of plants would better predict belowground microbial distributions than plant species identity alone since more closely related plant species would be expected to share morphological and functional traits that are important for structuring soil microbial communities (Cantarel *et al*. 2015).

METHODS

Soil sampling

We collected 625 surface soil cores (6.25 cm diameter × 20 cm depth) from the 0.5 km^2 Barro Colorado Island (BCI) forest dynamics plot, Republic of Panama (9.15° N, 79.8° W) (Hubbell & Foster 1983) between 14 October and 2 December 2010. Cores were sampled 1 m west of the central 5 m grid marker in every other 20 × 20 m quadrat. If there was an obstruction that precluded sampling in this location, we sampled at a nearby location. We sampled even columns on odd rows and odd columns on even rows of the 20 m grid system, resulting in *c*. 28 m spacing between nearest neighbour cores. Although our sampling scheme provided far higher spatial resolution than nearly any comparable study, we acknowledge that, by collecting soil cores spaced 28 m from one another, we may still lack the spatial resolution to capture detailed above–belowground relationships, given the heterogeneous nature of tropical forests and the soils at this site. We georeferenced the soil cores at all sites, using a differential GPS parameterised affine function (Wolf *et al*. 2015). Locations of the cores on both the plot coordinate system and a geographic coordinate system (WGS 1984 UTM Zone 17N, EPSG: 32617) are available in Wolf *et al*. (2015).

From each sampled location, we collected mineral soil (0–20 cm), excluding litter and organic horizons, using bulk soil rather than just rhizosphere soil for subsequent molecular analyses of microbial communities. Immediately after collecting the soil core, we placed the entire core in a Ziploc bag, thoroughly mixed the soil, and subsampled *c*. 15 mL of homogenised soil (no roots) into a Whirl-pak bag for microbial analyses. Soils were frozen at −20 °C on the same day of collection and were subsequently transported to Barnard College, Columbia University where they were stored at −20 °C until analysis. Prior to molecular analyses, we sieved soils through sterilised 2 mm sieves to homogenise the microbial community and remove any remaining rocks and non-soil fragments.

From the remaining soil, we collected roots for molecular analysis and subsampled 50 g of soil for air-drying and subsequent soil chemical analyses, which are described in Wolf *et al*. (2015). Briefly, we analyzed $BaCl_2$-extractable cations, and P from Mehlich-3 extractions, total carbon and total nitrogen, and soil pH in both H_2O and 0.1 M $CaCl_2$ solution.

Molecular analyses of microbial communities

Microbial diversity was assessed using high-throughput sequencing methods to characterise the variation in taxonomic marker gene sequences. For bacterial analyses, we sequenced the V4 hypervariable region of the 16S rRNA gene, using the 515-F (GTGCCAGCMGCCGCGGTAA) and 806-R (GGACTACHVGGGTWTCTAAT) primer pair (Fierer *et al*. 2012). Although this primer pair also captures Archaea, the number of 16S rRNA reads from Archaea was very low in this data set (< 2.2% of the total number of phylotypes and < 1.9% of total 16S rRNA sequences). For the fungal analyses, we sequenced the first internal transcribed spacer (ITS1) region of the rRNA operon, using the ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS2 (GCTGC GTTCTTCATCGATGC) primer pair (McGuire *et al*. 2013). The primers included the appropriate Illumina adapters with the reverse primers also having an error-correcting 12-bp barcode unique to each sample to permit multiplexing of samples.

PCR products from all samples were quantified using the PicoGreen dsDNA assay, and pooled together in equimolar concentrations for sequencing on an Illumina MiSeq instrument running the 2 × 150 bp chemistry. All sequencing runs were conducted at the University of Colorado Next Generation Sequencing Facility.

The forward reads were demultiplexed using a custom Python script (https://github.com/leffj/helper-code-for-uparse), with quality filtering and phylotype clustering conducted using the UPARSE pipeline (Edgar 2013). For quality filtering, we used a maxee value of 0.5 (indicating that on average a maximum of 0.5 nucleotides were incorrectly assigned in every sequence). Sequences were also dereplicated and singleton sequences were removed prior to phylotype determinations. Representative sequences from the phylotypes that were not ≥ 75% similar to sequences contained in either the Greengenes 13_8 database (McDonald *et al*. 2012) or the UNITE May, 2014 database (Abarenkov *et al*. 2010) for 16S and ITS rRNA sequences respectively, were discarded. Raw sequences were then mapped to phylotypes at the 97% similarity threshold. Phylotype taxonomy was determined using the RDP classifier with a confidence threshold of 0.5 (Wang *et al*. 2007) trained on the respective databases for 16S and ITS rRNA sequences. Sequences representing any phylotypes classified as mitochondria or chloroplast were removed. In order to reduce potential amplicon sequencing biases, we first removed samples with < 10 000 sequences and then we normalised the sequence counts, using a cumulative-sum scaling approach (Paulson *et al*. 2013). The total number of samples included in downstream analyses was 556 for bacteria and 480 for fungi. Representative sequences, phylotype abundance tables, and corresponding sample information are publicly available in FigShare (http://figshare.com/articles/Soil_microbial_communities_Barro_Colorado/1449286).

Determination of plant distributions

Aboveground stem distributions were determined from the 2010 BCI census. Every woody and palm stem ≥ 1.0 cm diameter at breast height (DBH) was sampled. Tree community composition was determined for five neighbourhood sizes (i.e. at 2.5, 5, 10, 15, and 20 m radii from the soil cores sampling points). Lianas were excluded from subsequent analyses. Palms were included in all analyses and, while we recognise that they are monocots and not woody trees, we use the term 'tree' throughout the paper for ease of distinguishing canopy and subcanopy plant species from understory plant species (which were not included in the current study).

We assessed belowground root distributions so we could simultaneously determine the relationships between plant aboveground or belowground distributions and soil microbial distribution patterns. We did this by conducting molecular analyses of bulk fine root samples that were separated from individual soil cores after washing the soil through a 0.1 mm sieve. We stored the fine roots from individual soil cores in a Tris-Low EDTA (TLE) buffer solution until dehydration by oven drying at 37 °C and homogenisation by mortar and pestle with liquid nitrogen. We used *c*. 0.1 g of the homogenised root tissue to extract DNA using a modified CTAB DNA extraction technique (Li *et al*. 2007). We removed PCR inhibitors from DNA extracts using a standard bead-based DNA cleanup with 1.8× AMPure substitute (Rohland & Reich 2012). Following DNA cleanup, we amplified root DNA using sequence-tagged *rbcL* primers (Kress *et al*. 2009). We visually confirmed amplification success by running fragments on 1.5% agarose and we normalised amplicons across plates by adding 10 μL of PCR product to SequalPrep Normalisation Kits (Invitrogen, Inc., Carlsbad, CA USA) using the standard protocol. After normalisation, we combined 6 μL from each normalised sample in a single tube, concentrated the combined DNA in a SpeedVac, and rehydrated the concentrated DNA with 16 μL 10 mM Tris-HCl. To apply sequence tags and sequencing adapters to each amplicon, we end-repaired and adenylated the rehydrated PCR amplicons by adding 16 μL of each amplicon pool to 1 μL End Prep Enzyme Mix (New England Biolabs, Ipswich, MA USA), 2.5 μL reaction buffer, and 5.5 μL ddH_2O. We incubated this reaction for 25 min at 25 °C followed by 20 min at 72 °C. We added 1 μL of 25 mM 454 sequencing adapters (indexed) to the pool of normalised amplicons along with 1 μL Quick T4 DNA ligase (New England Biolabs), and we incubated the ligation reaction for 10 min at 25 °C followed by 10 min at 65 °C. Following ligation, we added 38 μL of ddH_2O to each reaction along with 48.6 AMPure XP beads (Beckman Coulter Inc., Indianapolis, IN USA), and cleaned reactions following the standard AMPure protocol. We used qPCR to quantify each pool of amplicons, and we prepared an equimolar mixture of 1.0E8 copies by combining pools of amplicons together, and checked the size distribution of amplicons, using an Agilent BioAnalyzer (Santa Clara, CA USA) prior to sequencing. We sequenced the equimolar pools using either the Roche 454 FLX (1/8 lane) or the 454 Jr. (2 runs) at the UCLA Genotyping Core (Los Angeles, CA USA).

Prior to classifying sequences to species, we created a composite reference database by combining sequences from the database of BCI trees from Kress *et al*. (2009). We supplemented the composite reference database with sequences from Genbank for those species that occur on BCI but were absent from Kress *et al*. (2009). To classify sequence reads to woody plant species, we first removed reads shorter than 400 bp from the data set. Then we removed core samples having fewer than 30 total sequence reads, which we believe represented *rbcl* amplification failures. Following this filtering, we performed a BLAT search of all sequence reads to all species in the composite database, using a 98% similarity cutoff. We assigned a sequence read to a given reference species by minimising the number of base pair mismatches between the sequence read and reference data. Because *rbcL* is not completely diagnostic for all woody plant species on the BCI plot (Kress *et al*. 2009), we assigned ambiguous sequences (i.e. sequences with more than one matching species and the same number of base pair mismatches) to species in the reference database by computing the relative abundance of the potential matches in the plant neighbourhood within a distance of 15 m from the location of the soil core and assigning species identity based upon the potential match having the highest relative abundance (sum of total basal area of all stems > 1 cm of the candidate species). We selected 15 m as our radius because

this value is the maximum estimated crown radius for individuals in the neighbourhood around each sampling point (see below). If none of the potential matches were present within the 15 m neighbourhood, we removed the ambiguous sequence from the analysis. We also removed singletons (i.e. species with only one sequence present in a core) from the data set. The total number of root samples included in downstream analyses was 536.

Plant functional traits and phylogeny

We obtained the following plant functional traits for 248 of the 288 tree species (all woody and palm species ≥ 1.0 cm DBH) found in the 20 m neighbourhoods surrounding the soil cores: wood density, fruit mass, seed mass, average DBH, growth rate, mortality rate, leaf morphology (leaf area, leaf thickness, leaf mass per area), and leaf elemental chemistry (concentration of aluminum, calcium, potassium, magnesium, phosphorous, nitrogen and carbon) (Wright *et al*. 2010). For the 248 tree species for which we had trait information, phylogenetic relationships among these species were estimated according to a maximum-likelihood phylogeny (Kress *et al*. 2009). After multiple sequence alignment using MAFFT (Katoh & Standley 2013) and trimming of poorly aligned positions using GBLOCKS (Castresana 2000), phylogenetic relationships from root samples were estimated with the FastTree approximate maximum-likelihood algorithm (Price *et al*. 2010) and the midpoint method for rooting.

The distance between plant assemblages as determined from their trait distributions was calculated using the abundance weighted mean pairwise distance (MPD). MPD is defined as the average functional distance separating two species drawn at random from different assemblages (Webb 2000). Plant phylogenetic assemblage distance was calculated using the abundance weighted UniFrac metric, which is defined as percent of branch length unique to any pair of assemblages (Lozupone & Knight 2005). Functional trait and phylogenetic analyses were carried out in the R environment (www.r-project.org) using the ape (http://ape-package.ird.fr/) and picante (http://picante.r-forge.r-project.org/) packages.

Statistical analyses

Patterns in plant and microbial community similarity were represented by non-metric multidimensional scaling (NMDS) using the Bray–Curtis distance metric after Hellinger standardisation. We used partial Mantel tests controlling for the potential confounding effects of topography (slope) and soil pH as well as quantile regressions as implemented in the R package quantreg (https://cran.r-project.org/web/packages/quantreg/) to determine the relationships between plant and microbial community distance matrices. To estimate the explanatory power of individual soil and topographical variables on soil microbial communities and tree assemblages, we used permutational multivariate analysis of variance (PERMANOVA). Multivariate statistical analyses were implemented using the R packages vegan (http://vegan.r-forge.r-project.org/) and ecodist (http://cran.r-project.org/web/packages/ecodist/).

RESULTS AND DISCUSSION

Characteristics of the soil microbial communities at BCI

A total of of 33 480 bacterial and 24 610 fungal phylotypes were detected across all samples. Each soil sample collected from the 50-ha plot contained an average of 2600 bacterial phylotypes and 600 fungal phylotypes (Fig. S1). For trees, the average number of aboveground species associated with each soil sample was 6, 17, 42, 64, and 83 at increasing neighbourhood distances (2.5, 5, 10, 15 and 20 m radius, respectively) away from each soil sampling location, with a total of 288 trees species counted across all samples. On average, we identified roots from nine tree species in each soil core, with a total of 203 tree species detected in all soil cores (Fig. S1). We observed no significant relationship between patterns in plant and belowground microbial richness levels across the 0.5 km^2 plot (Fig. S2).

At the phylum level, the soil bacterial communities were dominated by Proteobacteria, Acidobacteria and Verrucomicrobia (34, 21, and 9% of 16S rRNA sequences respectively). The fungal communities were predominately composed of taxa within the Ascomycota and Basidiomycota phyla (66 and 27% of the fungal ITS1 sequences respectively) (Fig. S3). Tree species from the orders Gentianales (20%), Malpighiales (18%) and Sapindales (15%) dominated the aboveground samples, while Gentianales (23%), Rosales (16%), Fabales (14%) and Malpighiales (10%) dominated the belowground samples (Fig. S3).

Soil microbial community composition is correlated with aboveground tree distributions

For both soil bacteria and fungi, community similarity was weakly related to the geographic location of the sample (Fig. S4). As has been demonstrated previously (John *et al*. 2007), tree assemblage patterns were correlated with topography and soil characteristics (Fig. S5), and some of these same factors were also predictive of fungal and bacterial community composition (Fig. S5). In particular, tree community composition was associated with slope (PERMANOVA: $R^2 = 0.05$, $P < 0.001$), while microbial community composition was associated with soil pH. Bacterial communities showed a stronger relationship with soil pH than the fungal communities (PERMANOVA: $R^2 = 0.16$, $P < 0.001$ and $R^2 = 0.06$, $P < 0.001$, respectively; Fig. S5). These results are in line with other studies suggesting that the composition of soil bacterial communities, and to a lesser degree soil fungal communities, can be strongly influenced by differences in soil pH (Prober *et al*. 2015).

Both bacterial and fungal community distance patterns were positively associated with aboveground tree assemblage distance patterns (spatial visualisation of ordination axes in Figs 1, 2 and S6). We observed this positive relationship between soil microbial communities and tree assemblages after controlling for the potential confounding effects of shared

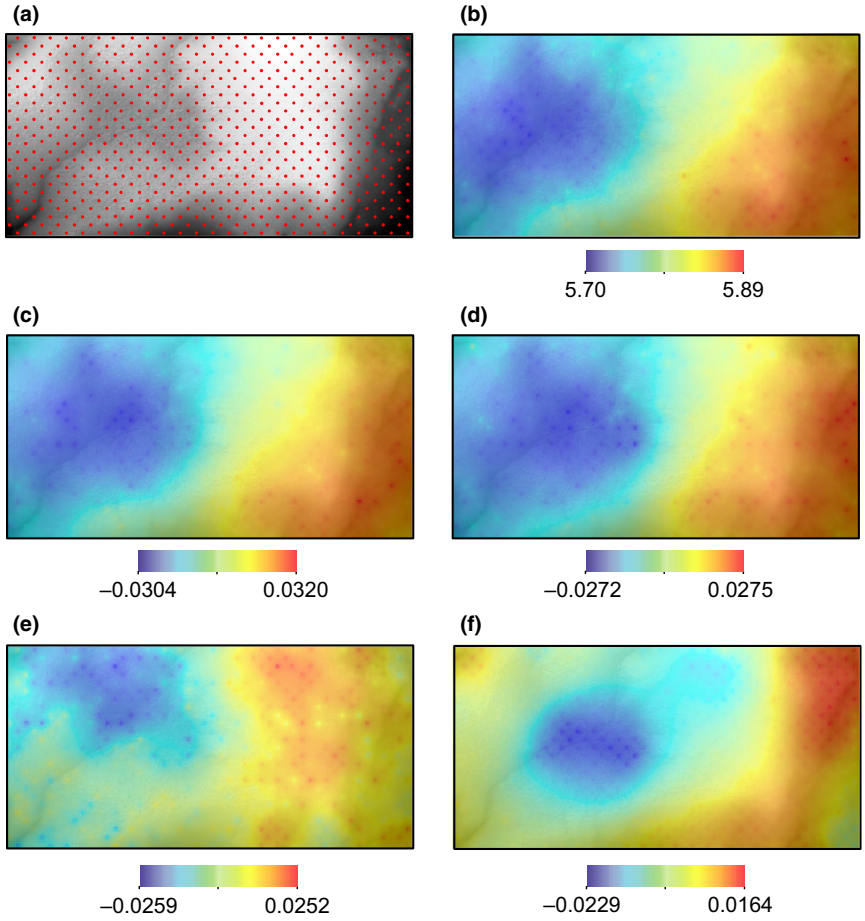

Figure 1 Sampling sites, soil pH, and maps of community similarity (NMDS axis scores). (a) Sampling sites for 625 soil cores (the 50 ha plot was divided into 20 × 20 m quadrats). (b) Soil pH. (c) Soil bacterial communities. (d) Soil fungal communities. (e) Root assemblages. (f) Tree assemblages. Interpolated values were calculated using inverse distance weighting with square-root distance.

environmental and habitat correlations by including topography (slope) and soil pH as covariates (Fig. 2). Thus, the correlations between tree distributions and belowground microbial community composition were not just driven by shared responses to changes in soil characteristics and topography across the plot. Rather, our data suggest that the observed correlations between the plant and soil microbial communities are related to aboveground-belowground feedbacks (Bever 2003), acknowledging that unmeasured environmental parameters or other biotic interactions might also be contributing to the observed patterns. When we re-ran these same models using only the largest trees in the plot (only those 27 tree species that had DBH values > 750 mm), we found weaker associations ($r = 0.13$ and 0.16 for the largest trees at 20 m neighbourhood compared to $r = 0.18$ and 0.27 for the whole data set at 20 m neighbourhood, for bacteria and fungi respectively). These results demonstrate that the correlations between soil microbial communities and the aboveground tree distributions are not just driven by the largest trees in the neighbourhood analyses.

Fungal community composition showed a stronger relationship with aboveground tree distributions than the bacterial communities (Figs 2 and S6), which is likely due to physiological and ecological differences between these groups (Waring *et al.* 2013). Fungi tend to depend more directly on plant products such as structural leaf litter compounds and root exudates (Broeckling *et al.* 2008) and are key decomposers of plant necromass (Boddy *et al.* 2008). Some groups of fungi also display resource-use specialisation on individual organic C and N compounds found in leaf litter (McGuire *et al.* 2010). Surprisingly, the relationship between arbuscular mycorrhizal fungi (Glomeromycota) and aboveground tree community composition was weaker than the one observed for overall fungal community composition ($r = 0.11$, $P < 0.01$ for Glomeromycota compared to $r = 0.27$, $P < 0.01$ for all fungi).

When we compared the aboveground plant assemblage data at different spatial neighbourhood sizes (2.5, 5, 10, 15 and 20 m), we found that the correlations between aboveground tree community composition and bacterial or fungal community composition were strongest at the largest neighbourhood size (20 m) (Figs 2 and S6). In other words, if we want to predict what types of bacteria or fungi we will find in an individual soil sample, it is most useful to know what trees are within a 20 m radius of that sample. It was somewhat surprising that the identity of trees very close to the soil sample was not nearly as useful for predicting microbial composition, because we would expect leaf litter inputs to be highest by trees closer to the sampling site. Our finding that the 20 m

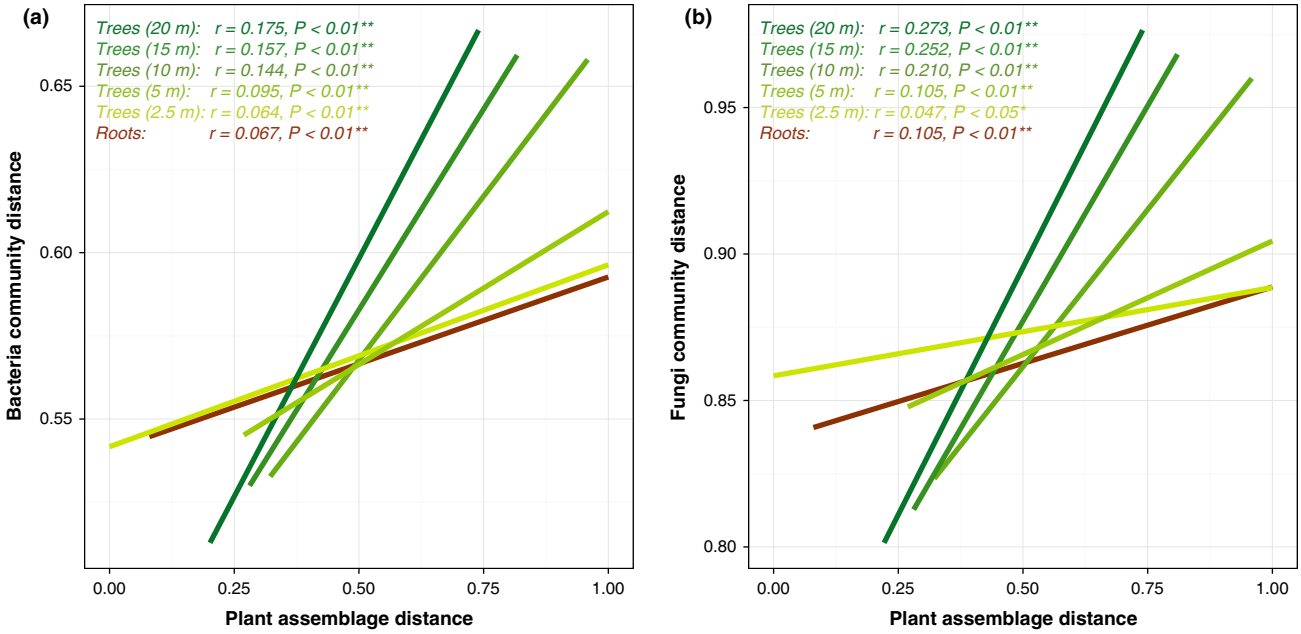

Figure 2 Relationships between (a) soil bacterial communities and (b) soil fungal communities with tree assemblages at increasing spatial scales and root assemblages. Differences among assemblages and communities estimated using Bray–Curtis distances. Lines represent fitted linear regressions between tree assemblages and associated bacterial or fungal communities. Statistics correspond to rank-based partial Mantel tests controlling for differences in slope and soil pH with *P*-values corrected for multiple comparisons by false discovery rate. Note the difference in *y*-axis scales. Similar results were obtained using quantile regression analyses (Fig. S6).

neighbourhood was more closely related to microbial composition suggests that plant community information collected at that scale may more effectively capture those plants that could be influencing soil at a given site. It may also be that non-additive litter mixture effects due to canopy overlap structure soil microbes in ways that are not predictable from single-species litter effects (Chapman *et al.* 2013). Additionally, differences in phenology (in particular, litter fall) may be another potential explanation for the observed relationships between tree assemblages and soil microbial community composition. For example, a recent study found that variation in litter inputs explained a significant proportion of soil C : N variability and that particular tree species dominated the litter inputs with seasonal differences (Uriarte *et al.* 2015). While litter phenology has not been explicitly evaluated on BCI, variability in species-specific litter phenology could explain the lack of a tight correlation between plant and microbial composition at smaller neighbourhood scales, particularly if litter from a more productive, neighbouring tree species was more influential on microbial composition at a given sampling point and time.

While phylogenetic relationships have been useful for explaining community assembly patterns for a variety of organisms (Cavender-Bares *et al.* 2009), and phylogenetic information can help explain some of the variation in some leaf-associated microbial communities (Kembel *et al.* 2014), incorporating phylogenetic information did not improve our ability to predict soil microbial distributions better than knowing species identity, alone (Figs 3 and S7). One ossible explanation for this finding is that the high taxonomic diversity of trees in the plot obscured the relative effects of phylogenetic vs. taxonomic distance, since these indices were highly correlated ($r = 0.86$, $P < 0.001$). Alternatively, the plant traits that shape belowground microbial communities may not be predictable from plant phylogeny due to convergence in selected phenotypic characteristics (Kursar *et al.* 2009).

Root distributions are poor predictors of microbial community composition

While we found fairly strong relationships between aboveground tree composition and soil microbial communities, there were only weak correlations between root distributions and microbial community composition (Figs 1, 2 and S6), which conflicts with our original prediction. This weak correlation was maintained when we conducted phylogenetic analyses of the root communities ($r = 0.061$, $P < 0.001$; $r = 0.128$, $P < 0.001$, for bacteria and fungi, respectively). Focusing solely on the composition of Glomeromycota did not improve the correlation with root composition ($r = 0.11$, $P = 0.11$). This result may be due to the fact that the distributions of aboveground trees and their roots are not well correlated at this site (Jones *et al.* 2011), which implies that either aboveground or belowground species-specific effects are spatially decoupled or that species-specific root zones may not actually exist like they do for aboveground neighbourhoods. There is some evidence for the latter hypothesis from a recent temperate forest study which found that roots from multiple tree species migrated to nutrient-enriched soil patches and diminished belowground species segregation across the forest (Valverde-Barrantes *et al.* 2015).

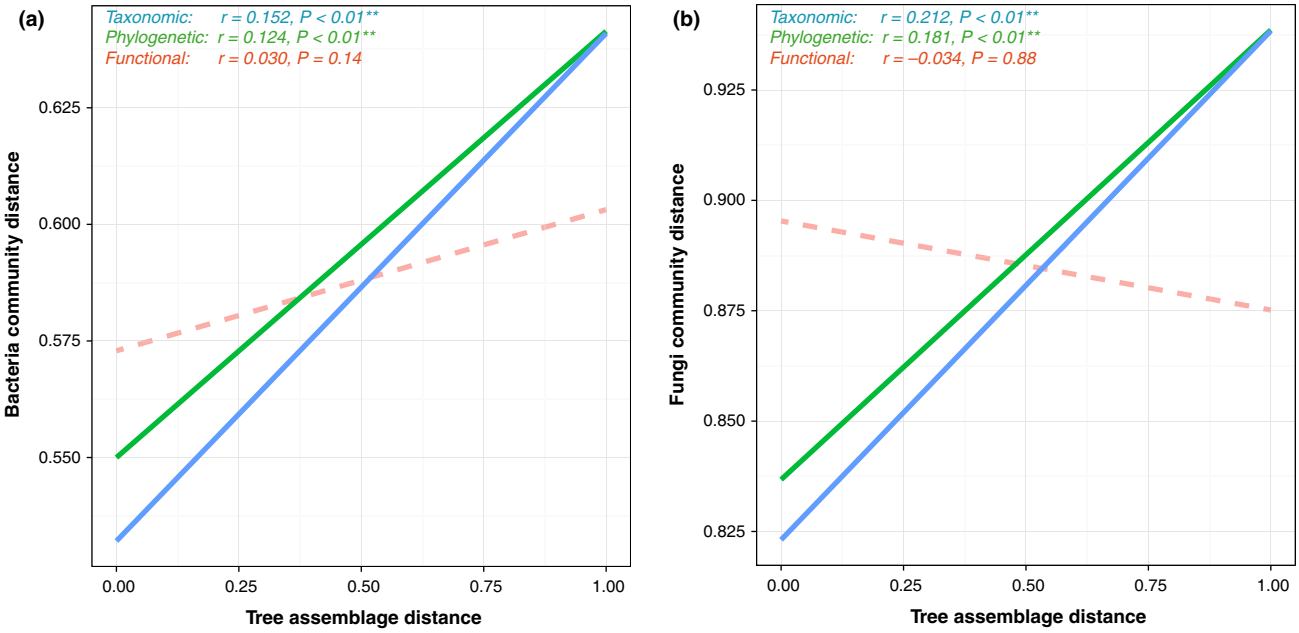

Figure 3 Relationships between (a) soil bacterial communities and (b) soil fungal communities with tree assemblage distance at the 20 m neighbourhood radius around each soil core based on taxonomic, phylogenetic and functional trait information. Only the species for which we had trait information were included in the analyses. Lines represent fitted linear regressions between assemblage distances and microbial community distances. Statistics correspond to rank-based partial Mantel tests controlling for differences in slope and soil pH with *P*-values corrected for multiple comparisons by false discovery rate. Dashed lines represent non-significant relationships. Note the difference in *y*-axis scales. Similar results were obtained using quantile regression analyses (Fig. S7).

Another explanation for the weak relationship between root and microbial composition is that roots are highly mobile and root distributions change quickly over time. Thus, temporal shifts in microbial communities may be uncoupled from the corresponding temporal dynamics of roots at this site. The average rate of fine root turnover for tree species in an adjacent forest was ~ 120 days, although significant species-specific variation likely exists (Yavitt *et al.* 2009). In addition, the microbes that are likely to be most affected by root dynamics may be rhizosphere specialists living on or around root surfaces rather than the microbes residing in bulk soil. Numerous studies have found that rhizosphere microbial communities are distinct from bulk soil microbial communities (Prescott & Grayston 2013), so species-specific effects of tree roots may be realised at much smaller spatial scales in the rhizosphere than could be detected from the bulk soil analyses conducted here. More manipulative studies are clearly necessary to unravel these alternative explanations, as most studies tend to have an aboveground bias and very little work has been done on the distributions of tropical tree roots (Iversen 2014).

Are tree traits predictive of soil microbial community composition?

While the taxonomic and phylogenetic metrics of tree assemblage composition were correlated with belowground bacterial and fungal communities, the aboveground leaf and stem traits were not good predictors of microbial community composition. Overall functional assemblage distance based on traits, using the abundance weighted mean pairwise functional distance (Webb 2000) was not correlated with soil microbial community composition (Figs 3 and S7). Given that there is a growing body of literature, suggesting that plant trait information is useful for predicting community assembly and ecosystem services (De Deyn & Van der Putten 2005; Cornwell *et al.* 2008), we were surprised to find that most plant trait distributions were not well-correlated with microbial distributions across the plot. However, it must be noted that most of the work linking microbial communities and microbially mediated processes to plant traits has been done in temperate grassland ecosystems (Cantarel *et al.* 2015), and it is possible that the patterns and processes documented in herbaceous ecosystems may not be observed in diverse tropical forests. It is also possible that other unmeasured traits, such as litter carbon chemistry or root traits (Cantarel *et al.* 2015), are more important drivers of microbial composition than the traits included in our analyses.

We also explored specific relationships between individual assemblage-weighted functional traits and soil bacterial or fungal community composition. Although we did not observe significant relationships between the overall, aggregated aboveground traits of the plant assemblages and the composition of the soil microbial communities, several individual traits showed weak correlations (Fig. S8). Specifically, Al content in leaves and tree mortality rate were correlated with both bacterial and fungal community composition. The relationship with Al may reflect the toxicity of that element to microbial cells (Pina & Cervantes 1996). For fungal communities, carbon and Ca content in leaves were also correlated with community composition (Fig. S8), which may be related to

the effects of these plant traits on soil C and litter input quantities. The relationship of leaf Ca concentrations and fungal composition likely reflects the important role of Ca in fungal growth, nutrient uptake and mycorrhizal symbiosis (Pera & Callieri 1997).

CONCLUSIONS

Despite the high tree diversity, we found significant correlations between tree composition, soil pH and the composition of soil microbial communities at the local scale. We also found correlations between microbial composition and a subset of assemblage-weighted plant traits. The lack of strong relationships between soil microbial composition and root distributions was surprising, but may be related to the ephemeral and mobile nature of fine roots. Since aboveground tree composition is less dynamic than belowground fine root turnover, the integrated effects of decadal litter inputs may be a more significant factor structuring soil microbial communities than root inputs.

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AUTHORSHIP

A.B., K.L.M., and N.F. developed and framed research questions. J.A.W., F.A.J., S.J.W. B.L.T, K.L.M., N.F., S.P.H., and B.C.F. provided data used in this study and logistical support. A.E. helped with sample processing. Data analyses were led by A.B. with help from K.L.M., N.F. and J.A.W. The paper was primarily written by N.F., K.L.M., and A.B. with help from all co-authors.

REFERENCES

Abarenkov, K., Nilsson, R.H., Larsson, K.H., Alexander, I.J., Eberhardt, U., Erland, S. *et al.* (2010). The UNITE database for molecular identification of fungi - recent updates and future perspectives. *New Phytol.*, 186, 281–285.

Bardgett, R.D., Bowman, W.D., Kaufmann, R., & Schmidt, S.K. (2005). A temporal approach to linking aboveground and belowground ecology. *Trends Ecol. Evol.*, 20, 634–641.

Bever, J.D. (2003). Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytol.*, 157, 465–473.

Bever, J.D., Broadhurst, L.M., & Thrall, P.H. (2013). Microbial phylotype composition and diversity predicts plant productivity and plant-soil feedbacks. *Ecol. Lett.*, 16, 167–174.

Boddy, L., Frankland, J.C., & Van West, P. (2008). *Ecology of Saprotrophic Basidiomycetes*. Elsevier Ltd., London.

Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K., & Vivanco, J.M. (2008). Root exudates regulate soil fungal community composition and diversty. *Appl. Environ. Microbiol.*, 74, 738–744.

Cantarel, A.A.M., Pommier, T., Desclos-Theveniau, M., Diquélou, S., Dumont, M., Grassein, F. *et al.* (2015). Using plant traits to explain plant–microbe relationships involved in nitrogen acquisition. *Ecology*, 96, 788–799.

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.*, 17, 540–552.

Cavender-Bares, J., Kozak, K.H., Fine, P.V.A., & Kembel, S.W. (2009). The merging of community ecology and phylogenetic biology. *Ecol. Lett.*, 12, 693–715.

Chapman, S.K., Newman, G.S., Hart, S.C., Schweitzer, J.A., & Koch, G.W. (2013). Leaf litter mixtures alter microbial community development: mechanisms for non-additive effects in litter decomposition. *PLoS ONE*, 8, e62671.

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O. *et al.* (2008). Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecol. Lett.*, 11, 1065–1071.

De Deyn, G.B. & Van der Putten, W.H. (2005). Linking aboveground and belowground diversity. *Trends Ecol. Evol.*, 20, 625–633.

Edgar, R.C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods*, 10, 996–998.

Fierer, N. & Lennon, J.T. (2011). The generation and maintenance of diversity in microbial communities. *Am. J. Bot.*, 98, 439–448.

Fierer, N., Leff, J.W., Adams, B.J., Nielsen, U.N., Bates, S.T., Lauber, C.L. *et al.* (2012). Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proc. Natl Acad. Sci. USA*, 109, 21390–21395.

van der Heijden, M.G.A., Bardgett, R.D., & van Straalen, N.M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.*, 11, 296–310.

Hines, J., Megonigal, J.P., & Denno, R.F. (2006). Nutrient subsidies to belowground microbes impact aboveground food web interactions. *Ecology*, 87, 1542–1555.

Hubbell, S.P. & Foster, R.B. (1983). Diversity of canopy trees in a Neotropical forest and implications for conservation. In *Tropical Rain Forest: Ecology and Management*. (eds Sutton, S.L., Whitmore, T.C., Chadwick, A.C.). Blackwell Scientific, Oxford, UK, pp. 25–41.

Iversen, C.M. (2014). Using root form to improve our understanding of root function. *New Phytol.*, 203, 707–709.

Jiang, Y.M., Chen, C.R., Xu, Z.H., & Liu, Y.Q. (2012). Effects of single and mixed species forest ecosystems on diversity and function of soil microbial community in subtropical China. *J. Soils Sediments*, 12, 228–240.

John, R., Dalling, J.W., Harms, K.E., Yavitt, J.B., Stallard, R.F., Mirabello, M. *et al.* (2007). Soil nutrients influence spatial distributions of tropical tree species. *Proc. Natl Acad. Sci. USA*, 104, 864–869.

Jones, F.A., Erickson, D.L., Bernal, M.A., Bermingham, E., Kress, W.J., Herre, E.A. *et al.* (2011). The roots of diversity: below ground species

richness and rooting distributions in a tropical forest revealed by DNA barcodes and inverse modeling. *PLoS ONE*, 6, e24506.
Katoh, K. & Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.*, 30, 772–780.
Kembel, S.W., O'Connor, T.K., Arnold, H.K., Hubbell, S.P., Wright, S.J., & Green, J.L. (2014). Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proc. Natl Acad. Sci. USA*, 111, 13715–13720.
Kress, W.J., Erickson, D.L., Jones, F.A., Swenson, N.G., Perez, R., Sanjur, O. *et al.* (2009). Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. *Proc. Natl Acad. Sci. USA*, 106, 18621–18626.
Kursar, T.A., Dexter, K.G., Lokvam, J., Pennington, R.T., Richardson, J.E., Weber, M.G. *et al.* (2009). The evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus Inga. *Proc. Natl Acad. Sci. USA*, 106, 18073–18078.
Li, J., Yang, J., Chen, D., Zhang, X., & Tang, Z. (2007). An optimized mini-preparation method to obtain high-quality genomic DNA from mature leaves of sunflower. *Genet. Mol. Res.*, 6, 1064–1071.
Lozupone, C. & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.*, 71, 8228–8235.
McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A. *et al.* (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J.*, 6, 610–618.
McGuire, K.L., Bent, E., Borneman, J., Majumder, A., Allison, S.D., & Treseder, K.K. (2010). Functional diversity in resource use by fungi. *Ecology*, 91, 2324–2332.
McGuire, K.L., Fierer, N., Bateman, C., Treseder, K.K., & Turner, B.L. (2012). Fungal community composition in Neotropical rain forests: the influence of tree diversity and precipitation. *Microb. Ecol.*, 63, 804–812.
McGuire, K.L., Payne, S.G., Palmer, M.I., Gillikin, C.M., Keefe, D., Kim, S.J. *et al.* (2013). Digging the New York City skyline: soil fungal communities in green roofs and city parks. *PLoS ONE*, 8, e58020.
Paulson, J.N., Stine, O.C., Bravo, H.C., & Pop, M. (2013). Differential abundance analysis for microbial marker-gene surveys. *Nat. Methods*, 10, 1200–1202.
Pera, L.M. & Callieri, D.A. (1997). Influence of calcium on fungal growth, hyphal morphology and citric acid production in Aspergillus niger. *Folia Microbiol.*, 42, 551–556.
Pina, R.G. & Cervantes, C. (1996). Microbial interactions with aluminium. *Biometals*, 9, 311–316.
Prescott, C.E. & Grayston, S.J. (2013). Tree species influence on microbial communities in litter and soil: current knowledge and research needs. *For. Ecol. Manage.*, 309, 19–27.
Price, M.N., Dehal, P.S., & Arkin, A.P. (2010). FastTree 2-approximately maximum-likelihood trees for large alignments. *PLoS ONE*, 5, e9490.
Prober, S.M., Leff, J.W., Bates, S.T., Borer, E.T., Firn, J., Harpole, W.S. *et al.* (2015). Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecol. Lett.*, 18, 85–95.
Rohland, N. & Reich, D. (2012). Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Res.*, 22, 939–946.
Talbot, J.M., Bruns, T.D., Taylor, J.W., Smith, D.P., Branco, S., Glassman, S.I. *et al.* (2014). Endemism and functional convergence across the North American soil mycobiome. *Proc. Natl Acad. Sci. USA*, 111, 6341–6346.
Uriarte, M., Thompson, J., Turner, B.L., & Zimmerman, J.K. (2015). Linking spatial patterns of leaf litterfall and soil nutrients in a tropical forest: a neighborhood approach. *Ecol. Appl.*, 25, 2022–2034.
Valverde-Barrantes, O.J., Smemo, K.A., Feinstein, L.M., Kershner, M.W., & Blackwood, C.B. (2015). Aggregated and complementary: symmetric proliferation, overyielding, and mass effects explain fine-root biomass in soil patches in a diverse temperate deciduous forest landscape. *New Phytol.*, 205, 731–742.
Wang, Q., Garrity, G.M., Tiedje, J.M., & Cole, J.R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.*, 73, 5261–5267.
Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H., & Wall, D.H. (2004). Ecological linkages between aboveground and belowground biota. *Science*, 304, 1629–1633.
Waring, B.G., Averill, C., & Hawkes, C.V. (2013). Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: insights from meta-analysis and theoretical models. *Ecol. Lett.*, 16, 887–894.
Webb, C.O. (2000). Exploring the phylogenetic structure of ecological communities: an example for rain forest trees. *Am. Nat.*, 156, 145–155.
Wolf, J.A., Hubbell, S.P., Fricker, G.A., & Turner, B.L. (2015). Geospatial observations on tropical forest surface soil chemistry. *Ecology*, 96, 2313–2313.
Wright, S.J., Kitajima, K., Kraft, N.J.B., Reich, P.B., Wright, I.J., Bunker, D.E. *et al.* (2010). Functional traits and the growth-mortality trade-off in tropical trees. *Ecology*, 91, 3664–3674.
Yavitt, J.B., Harms, K.E., Garcia, M.N., Wright, S.J., He, F., & Mirabello, M.J. (2009). Spatial heterogeneity of soil chemical properties in a lowland tropical moist forest, Panama. *Aust. J. Soil Res.*, 47, 674–687.

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