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REGULAR ARTICLE

Differences in the soil microbiomes of *Pentaclethra macroloba* across tree size and in contrasting land use histories

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Abstract
Aims Legacy attributes from land-use history have lingering effects on soil and its below-ground components undergoing succession that has important consequences for regenerating tropical secondary forests. Yet, even landscapes of similar origins with analogous land-use histories have exhibited differing routes of forest recovery with different outcomes. There is increasing evidence that tree species-generated soil microbial heterogeneity is an important factor in facilitating regeneration, particularly nitrogen (N)-fixing tree species. However, it is unclear how land-use history influences the soil microbiome of important N-fixing plants developing under these conditions; at different life stages of N-fixing plant development; and how this compares to a primary forest.

Methods We examined differences in composition of the soil bacterial and fungal communities and their determinants (i.e. soil environmental factors) associated with large-, medium-, and small-sized *Pentaclethra macroloba* trees in a primary forest and in a 23-year-old secondary forest with contrasting land-use histories.
Results We show that as *Pentaclethra* increases in size (and/or age), the soil microbiome associated with *Pentaclethra* also changes, and that these soil microbiomes can become similar even when developed in soils of contrasting land-use histories. We found that soil NH_4^+ and NO_3^- explained 61% of the variation in the soil bacterial community composition of small trees between the primary and secondary forest.
Conclusions This highlights the importance of inorganic N during tree soil microbiome development in contrasting land-use history of soils. Our findings suggest that legacy effects on may be mediated through size (and/or age) of *Pentaclethra* and its associated soil microbiome.

Keywords 16S rRNA · ITS rRNA · *Pentaclethra macroloba* · Soil · Costa Rica · Zinke effects

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Introduction

Land-use histories in the tropics have resulted in complex interactions between the biotic and abiotic components of ecosystems, and consequently, the trajectory of forest regeneration can have multiple and unpredictable successional pathways. Indeed, even landscapes of

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similar origins with analogous land-use histories, or within a single tree species, have exhibited differing routes of forest recovery with different outcomes, often due to legacy effects (Kuusipalo et al. 1995; Leopold et al. 2001; Chaparro et al. 2012, 2014; Kashian et al. 2013; Norden et al. 2015). Legacy effects from land-use histories include lingering alterations to the soil structure, carbon (C), nitrogen (N), or other biogeochemical cycles, water flow, seed banks, the amount of light reaching the forest floor, amount of woody debris, and the forest flora and fauna (Hogan et al. 2016; Bürgi et al. 2016; Ziter et al. 2017) —causing significant depletions of the critical soil C and N components. As land-use impacts and soil disturbance regimes are likely to have an influence on individual plant effects (Hobbie 1992; Powers et al. 1997; Holl 1999; Gei and Powers 2013; Waring et al. 2015), plant-induced soil microbial heterogeneity will likely have important consequences as potential forces structuring regeneration pathways (Powers et al. 1997, 2009; Holl 1999; Chazdon et al. 2016).

In contrast to the two-way relationship of plant-soil feedbacks, individual plant 'Zinke' effects only concern the effects of the tree on the soils beneath and immediately adjacent to the tree (Zinke 1962). Individual plant effects are known to influence soil microbial communities (Wardle et al. 2004; Fierer et al. 2007; Ushio et al. 2008; Miki et al. 2010) that creates a zone of influence under the tree canopy (Zinke 1962) through the release of biochemicals and dead plant cells, and by providing photosynthate (Kimmins et al. 1990; Chaparro et al. 2012; Gougoulias et al. 2014; Lakshmanan et al. 2014); the degree of which differs among plant species and successional stage (Bauhus et al. 1998; Wardle 2002, 2006). Some work has demonstrated that individual plant effects influence soil abiotic properties and soil biotic community composition for temperate tree species (Zinke 1962; Hobbie 1992; Binkley and Giardina 1998), but less for tropical tree species (Ushio et al. 2008; McGuire et al. 2010; Raich et al. 2014); however, in neither case was there an explicit focus on recovery from disturbance. These individual plant effects on the belowground components may provide mechanistic links to the unexplained stochasticity in forest recovery following disturbance.

Soil microbial populations are susceptible to the altered stochastic processes that occur when normal plant and soil conditions are disturbed. This often facilitates the dispersal and invasion of other organisms which may have potential consequences for overall plant productivity and ecosystem function of secondary forest development (Richter et al. 2000; Foster et al. 2003). During the early stages of tree development, the soil bacterial and fungal groups that come together to form the soil microbiome of tree species, would likely be more susceptible to changes resulting from stochastic processes, as well as the presence of other groups of competing microbes (Brussaard 1997; Griffiths et al. 2000; van der Heijden et al. 2008; Allison and Martiny 2008; Fierer et al. 2010; Kardol and Wardle 2010; Kardol et al. 2013). Yet, even if priority effects and deterministic and stochastic processes act concurrently (Martiny et al. 2006; Nemergut et al. 2013; Barberán et al. 2015; Tedersoo et al. 2016; Fukami et al. 2017), it is unclear which soil abiotic properties may influence these soil microbial populations in a direction or trajectory in a disturbed area, such that it will become more similar to the original biotic community before the major disturbance.

Soils of primary old-growth forests in tropical lowland ecosystems are often highly weathered and typically described as relatively poor in soil phosphorus (P), yet rich in atmospherically-derived soil nitrogen (N) (Vitousek 1984; Vitousek and Sanford 1986; Vitousek and Howarth 1991; Guariguata and Ostertag 2001). However, land-use activities such as the conversion to agriculture, especially those involving slash and burn techniques, can cause deleterious losses of soil N. Consequently, these nutrient losses can drive the early stages of secondary succession toward N-limitation, that has potential negative feedbacks for not only plant growth but also for critical soil biomass development (Guariguata and Ostertag 2001; Feldpausch et al. 2004; Booth et al. 2005; Gehring et al. 2005; Sahrawat 2008; Siddique et al. 2010).

Indeed, one such set of biotic factors presumed to be important in ecosystem development of forests are the symbiotic N-fixing bacterial communities associated with the root nodules of N-fixing tree species. This biologically important plant attribute can ameliorate damaged soils through increases in the availability of soil N that is important for biosynthetic pathways in plant and soil microbial biomass development, which has critical implications for improved soil fertility (Hart et al. 1997; Guariguata and Ostertag 2001; Gehring et al. 2005; Nichols and Carpenter 2006; Eaton et al. 2012; Shebitz and Eaton 2013). *Pentaclethra macroloba* (Wiild.) Ktze (Fabaceae) is a dominant early colonizing

and dominant later stage leguminous forest canopy tree in the Northern Zone of Costa Rica, that is important in the recuperation of soil N and C cycle dynamics and microbial biomass enhancement during secondary forest succession (Hartshorn et al. 1994; Pons et al. 2006; Eaton et al. 2012; Shebitz and Eaton 2013). It is unclear how past land-use history may influence the soil biotic development associated with *Pentaclethra*, and what, if any, are the implications or consequences of this with respect to forest successional trajectory. The dearth of these types of studies can hinder our understanding of how previous land management influences the soil microbiome of important N-fixing plants developing under these conditions; at different life stages of N-fixing plant development; and how this compares to an undisturbed ecosystem such as a primary forest.

To begin to address this, we examined the soil bacterial and fungal community composition and various soil abiotic factors surrounding different size classes (as a proxy for age) of *Pentaclethra*, in a primary forest and in a secondary forest, and determined if there were differences in these soil microbial communities in each size class between the primary and secondary forest trees. Moreover, we wanted to investigate which soil abiotic properties were driving the differences in the soil microbiomes of *Pentaclethra* across tree-size and between the primary and secondary forest. It is possible there is a legacy affect after forest clearing that may have implications for the ecosystem in which *Pentaclethra* occurs, that may shape the soil microbial communities in different ways than a primary forest. For the current study, we asked three questions:

(1) Are there differences in the soil bacterial and fungal community composition between large, medium, and small sized *Pentaclethra*-trees, within a primary forest, and within a secondary forest?
(2) Are there differences in the soil bacterial and fungal community composition for each size class of *Pentaclethra* trees between a primary and secondary forest?
(3) Which soil abiotic factors are structuring the soil microbial communities in these different comparisons?

Here we provide the first characterization of plant 'Zinke' effects and evidence that as the *Pentaclethra* soil microbiome is initially dissimilar developing in a contrasting land-use history than a primary forest, the soil microbiome of *Pentaclethra* soils can become increasingly similar across tree-size even in contrasting land-use history of the same origin and soil texture.

Materials and methods

Site description

This study was conducted in the Maquenque National Wildlife Refuge (MNWR) (10°27'05.7"N, 84°16'24.32"W) (Fig. S1) of Costa Rica within two upland habitat types: a primary old-growth forest and a 23-year-old secondary forest. These two habitats were at one point in time, all a part of a single tract of primary forest with the same soil type (oxisol), topography, and soil textures but have been managed differently in the past ~40 years (total area 500 ha). The primary forest has not experienced anthropogenic disturbance in the known history of the area (personal communication, K. Schmack, Laguna del Lagarto Lodge). In contrast, the 23-year-old secondary forest was cut 33-years ago, used for cattle pasture for 10 years, and then allowed to regenerate. Thus, even though the past land-use histories are different between these two forested habitats, they both originated from the same forested landscape, soil topography, and soil texture (McGee et al. 2018, 2019). The mean annual temperature is 27° C, mean annual rainfall is 4300 mm, and the dominant soil type are oxisols (Hartshorn et al. 1994).

Soil sample collection

To address questions 1–3, large- ($\geq$ 30 cm DBH), medium- (5 cm $\leq$ DBH $\leq$ 10 cm), and small- ($\leq$ 1 cm) sized *Pentaclethra* trees in the upland regions of the primary forest and the secondary forest, were used for soil sample collection. We used tree-size (DBH) as a proxy for age as *Pentaclethra macroloba* does not form visible tree rings detectable by traditional dendrochronological methods. Size was used as a proxy for age because trees in this area do not form visible annual growth rings detectable by traditional dendrochronological tree core methods on intact trees (Enquist and Leffler 2001; Abrams and Hock 2006). This is due to an aseasonal with respect to temperature in this area. Six *Pentaclethra* large-, medium-, and small-trees per habitat were targeted, resulting in 18 *Pentaclethra* trees per habitat type, and six per size class. Trees were chosen at

least 20 m away from the edge of any trails, no slope aspect greater than 10%, and proximity to neighboring trees based on a tree protection zone (TPZ—described below) (McNulty and Barry 2009; Whiting 2013). The sampling area around the tree was based on the DBH of each tree so a tree protection zone (TPZ) radius could be calculated, as the size of the TPZ radius is relative to the DBH of a tree (Whiting 2013). To calculate the TPZ radius, formulas described by Whiting (2013) were applied (Fig. S2). This TPZ radius number provides the maximum distance away from the base of the tree in which to sample. However, to eliminate overlap with conspecifics and to minimize the influence of other neighboring plants, the maximum distance from tree was not examined. Instead, only three soil zones away from the tree were examined to assess individual plant effects (described below).

Using a compass, four transects were constructed in each cardinal direction from the base of the tree, and 10%, 20%, and 30% zones were determined (e.g. Fig. S2). The purpose of establishing soil sampling transects in each cardinal direction was to provide uniform sampling around the tree to obtain one representative composite soil sample per tree (Bruckner et al. 2000; Bélanger and Van Rees 2006; van der Gast et al. 2011; Whiting 2013). This approach to soil sampling was to reduce microsite variability given the heterogeneous properties in tropical soils (Enquist and Leffler 2001; Buckley and Schmidt 2003; Abrams and Hock 2006). One soil core (7.5 cm × 15 cm × 1.25 cm) was taken along the North, South, West, and East transects in the 10%, 20%, and 30% zones, yielding 12 soil cores per tree. These 12 soil cores per tree were bulked in sterile Whirl-Pak® (Nasco, Fort Atkinson, WI, USA) bags resulting in one composite soil sample per tree. Soil cores were taken aseptically (sterilized with 70% ethanol between each tree) to avoid cross contamination, minimize spatial autocorrelation, and ensure independent sampling. The effect of direction was not examined, and therefore, sterilizing the soil core and collection gloves between directions/transects was not necessary. A gardening punch (5 cm × 15 cm) was used to collect soil from the small (sapling) *Pentaclethra* trees in the primary forest and in the secondary forests. The sapling trees were small enough that the punch could be brought down around the tree and collect a core of soil with the sapling being in the middle of the core. For homogenization, all soil samples were mixed and passed through a 4-mm sieve using sterile techniques at field moist conditions prior to all downstream analyses. Elevation of the primary and secondary forest were measured with a Garmin Rino 650 (Garmin International, Olathe, KS, USA) GPS. The distance between the primary forest (10°40′46.21”N, 84°10′42.10”W) and the secondary forest (10°41′7.92“N, 84° 9’57.30”W) is approximately 1.5 km.

Soil abiotic properties

The soil abiotic properties measured were, % C (as ToC), %N (as TN), NH_4^+, NO_3^-, soil microbial biomass C (C_{mic}), *p*H, and % moisture, and have been described previously (McGee et al. 2018, 2019). Soil percent moisture and *p*H were measured at each soil core sample location at the time of soil sampling with a Kelway Soil *p*H and Moisture meter (Kel Instruments Co., Inc., Wyckoff, NJ, USA). The differences in the soil abiotic factors among the *Pentaclethra* tree-size comparison and between the primary trees and secondary trees forest can be found in the supplementary material, Tables S1-S3.

DNA extraction, sequencing, and bioinformatic analyses

Soil environmental microbial DNA was extracted from three 0.33 g replicate sub-samples for a total of 1 g for each soil sample using the MoBio PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA), following manufacturer’s protocol. The concentration and purity (A_{260}/A_{280} ratio) of extracted soil eDNA were determined prior to downstream analyses using a NanoDrop 1000 spectrophotometer (ThermoFisher Scientific, Waltham, MA). All eDNA was stored at −80 °C. PCR amplification of eDNA was performed targeting the V3 and V4 of 16S ribosomal RNA gene region for bacteria and archaea (Caporaso et al. 2011) and the nuclear ribosomal internal transcribed spacer (ITS) region for fungi (Gardes and Bruns 1993). One fragment of the 16S gene region was amplified by PCR targeting two non-overlapping variable gene regions v3 (~197 bp) and v4 (~288 bp) using one primer set 16Sv3F 5’-ACTCCTAC GGGAGCAGCAG-3′ and 16Sv4R 5’-GGACTACA RGGTATCTAAT-3′ (Sundquist et al. 2007). The variable ITS1 and ITS2 regions were amplified including the intercalary 5.8S rRNA gene (> 500 bp) using one primer set, ITS1F 5’-CTTGGTCATTTAGAGGAAGT

AA-3′ and ITS4 5’-TCCTCCGCTTATTGATATGC-3′ (White et al. 1990). Amplicons were prepared with a two-step PCR regime. The first step was performed with the primers listed above. Each PCR amplification contained 2 μL DNA template, 17.5 μL molecular biology grade water, 2.5 μL 10x reaction buffer (200 mM Tris-HCl, 500 mM KCl, pH 8.4), 1 μL 50x $MgCl_2$ (50 mM), 0.5 μL dNTPs mix (10 mM), 0.5 μL forward primer (10 mM), 0.5 μL reverse primer (10 mM), and 0.5 μL Invitrogen Platinum Taq polymerase (5 U/μL) in a total volume of 25 μL. The PCR conditions were 95 °C for 5 min; 35 cycles of 94 °C for 40 s, 46 °C for 1 min, and 72 °C for 30 s; and 72 °C for 5 min; and held at 4 °C. PCR products were visualized on 1.5% agarose gels to confirm successful amplification by the presence of fluorescent bands under a UV spectrophotometer. PCR products were then purified using a Qiagen MinElute PCR purification kit (Qiagen, Valencia, CA, USA) and eluted in 30 μL of molecular biology grade water. A second PCR step was implemented using 2 μl of the purified 1st PCR product as a template and with Illumina adaptor-tailed primers. The 2nd PCR was made following the same protocol as aforementioned except 30 cycles were used for PCR. All PCRs were done using Eppendorf Mastercycler ep gradient S thermalcyclers and negative control reactions (no DNA template) were included in all experiments. All generated soil amplicons plates were dual indexed and sequenced in several Illumina MiSeq runs using a V2 MiSeq sequencing kit (500 cycles – 250 bp × 2) (FC-131-1002 and MS-102-2003).

Bioinformatic methods have been described previously in McGee et al. (2019) and a more detailed description can be found in the supplementary material. All generated raw sequencing data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under the BioProject accession numbers: PRJNA526523 and PRJNA527065.

Statistical and multivariate analyses

To determine if significant differences were occurring between the mean values of the soil abiotic properties across size class, and between primary and secondary forests, values were compared by one-way analysis of variance (ANOVA), followed by Tukey HSD or Dunnett T3 post-hoc analysis where appropriate due to equal or unequal variances, and by independent samples t-test in SPSS (v.22, Armonk, NY, USA). Soil bacterial and fungal genera richness (d) (Margalef’s richness) and Shannon Index (H′) were calculated using Primer-E (Anderson et al. 2008). Significant differences among these mean values of alpha diversity indices for soil bacterial and fungal genera were also determined using a one-way ANOVA followed by post-hoc analyses in SPSS (v.22, Armonk, NY, USA) with either a Tukey HSD or a Dunnett T3 post-hoc, based on equal or unequal variances.

All multivariate analyses were performed in PRIMER-E v6 (Clarke and Gorley 2006) and its add-on PERMANOVA+ (Anderson et al. 2008). Prior to analysis, Draftsman plots (variable pair-wise scatter plots) were used to determine the homogeneity and multicollinearity of each soil abiotic variable (predictor variables). All environmental data were normalized and transformed using the log (x + 1) transformation to correct for skewness (Clarke and Gorley 2006; Anderson et al. 2008). The differences in the soil bacterial and fungal community compositions were analyzed separately. Prior to analysis, the soil bacterial and fungal community composition was 4th root transformed to account for dominant, as well as rare taxa, and then calculated into a Bray-Curtis Dissimilarity matrix. To test if the soil bacterial and fungal community composition was significantly different across size class within each site (primary and secondary forest), a 1-way permutational analysis of variance (PERMANOVA) was implemented with 2-factors: site and size, with size nested in site (question 1). To determine if the soil bacterial and fungal community composition was significantly different between primary and secondary forest for each size class (question 2), a 1-way permutational analysis of variance (PERMANOVA) was implemented with 2-factors: site and size, with site nested in size. All of these analyses were done using main and pair-wise tests based on unrestricted permutations (9999 permutations) on the fourth-root transformed soil bacterial and fungal community composition pairwise Bray-Curtis dissimilarity resemblance matrices (Clarke 1993). These analyses were nested to account for potential lack of independence between samples that were located at the same site. The PERMANOVA results of the soil biotic community composition among the various pairwise comparisons were considered significant, if $p \leq 0.05$.

A discriminant analysis using the Canonical Analysis of Principal Coordinates (CAP) was used to visualize the distinctiveness and how well the axes discriminate

among the groups (Anderson and Willis 2003) of the soil bacterial and fungal community composition across the different groups based on an a priori allocation success, using the PERMANOVA+ guidelines (Anderson and Willis 2003; Anderson et al. 2008). Strong differences between the groups are represented by CAP axis squared canonical correlations greater than or equal to 0.7 and moderate differences are represented by axis squared canonical correlations greater than or equal to 0.5–0.69 (Anderson and Willis 2003). Furthermore, Cohen's *d* effect sizes were calculated for the PERMANOVA pairwise comparisons to assess if the differences were trivial or not, and used as indicators of biologically meaningful differences between mean values of the parameters measured, as recommended for analysis of small sample sizes (DiStefano et al. 2005). The Cohen's *d* effect size statistics are considered small if $d = 0.2$, medium if $d = 0.5$–0.7, and large if $d \geq 0.8$.

To address question 3, a distance-based linear model (DistLM) approach was used to identify which soil environmental variables structure the multivariate patterns of the soil bacterial and fungal communities of the different *Pentaclethra* comparisons. The DistLM was performed using a 'step-wise' selection procedure and an AICc (Akaike's Information Criterion Corrected) selection criterion with 9999 permutations (Akaike 1978; Sakamoto et al. 1986; Legendre and Anderson 1999; Legendre et al. 2005; Anderson et al. 2008). This was based on the same biological 4th root transformed and resemblance matrices and log (x + 1) transformed environmental data (normalized). Distance-Based Linear Modeling marginal and sequential tests were considered significant, if $p < 0.05$. These DistLM results were then visualized using a Distance-Based Redundancy Analysis (dbRDA) in which the ordination is plotted from the values of the given model that best explains the greatest variation in the data cloud (Legendre and Anderson 1999; Anderson et al. 2008).

Results

Differences in the soil bacterial & fungal community composition

The most common soil bacterial taxa were Acidobacteria Groups, Verrucomicrobia, *Burkholderia*, and *Nitrospira* (Table S4) and the most common soil fungal taxa were *Mortierella*, *Trichosporon*, *Myxocephala*, *Archaeorhizomyces*, and *Geotrichum* (Table S5). The CAP results for the soil bacterial community composition across tree size within each habitat and between the habitat, was able to correctly allocate 28 out of the 36 (77.78%) soil samples to their original group, for a misclassification error of 22.22% out of 9999 permutations (Pseudo-F = 2.97, $p = 0.0001$) (Fig. 1a) (Table S6). The CAP results for the soil fungal community composition across tree size within each habitat and between the habitats, was able to correctly allocate 31 out of 36 (86.11%) soil to their original group, for a misclassification error of 13.89% out of

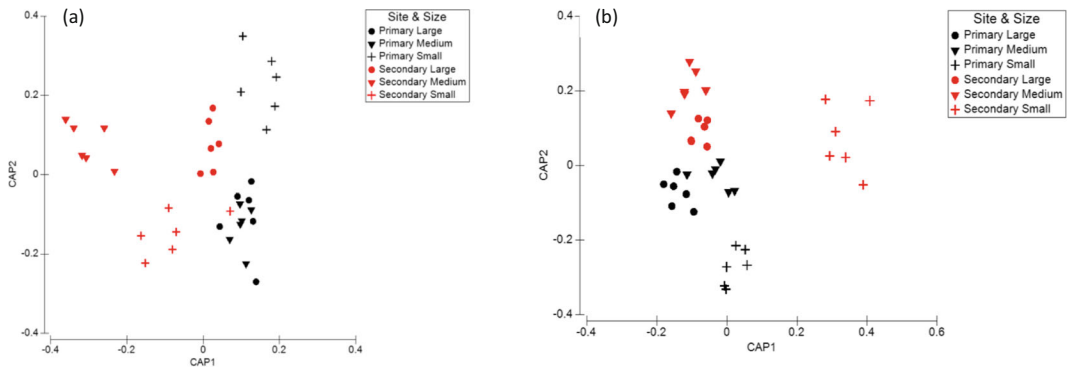

Fig. 1 The Canonical Analysis of the Principal Coordinates (CAP) showing the strength of the dissimilarity in the **a** soil bacterial community composition and **b** the soil fungal community composition across the large, medium, and small *Pentaclethra macroloba* tree-soils and their respective habitat, in the MNWR. For the soil bacterial community composition, the CAP was able to correctly allocate 28 out of the 36 (77.78%) soil samples to their original group for a misclassification error of 22.22% out of 9999 permutations (Pseudo-F = 2.97, $p = 0.0001$) (Table S6). For the soil fungal community composition, the CAP was able to correctly allocate 31 out of the 36 (86.11%) soil samples to their original group for a misclassification error of 13.89% out of 9999 permutations (Pseudo-F = 4.07, $p = 0.0001$) (Table S7)

9999 permutations (Pseudo-F = 4.07, $p = 0.0001$) (Fig. 1b) (Table S7).

The soil bacterial and fungal community composition was clustered by tree size within the primary forest, and within the secondary forest (pairwise comparisons, $p < 0.05$), except for the soil bacterial communities between the large tree-soils and the medium tree-soils in the primary forest ($p = 0.1317$) (Table 1). Moreover, the soil bacterial and fungal community composition per tree size, between the primary forest and the secondary forest, were significantly different (pairwise comparisons, $p < 0.05$) (Table 1), except for the soil bacterial community composition of large tree-soils between the primary and the secondary forest ($p = 0.1059$) (Table 2). Differences in the soil bacterial and fungal genus richness and diversity for all comparisons can be found in the supplementary material (Tables S8 and S9).

Determinants of the soil bacterial & fungal community composition

Large, medium, and small Pentaclethra tree-soils: within the primary forest and within the secondary forest

In the primary forest across the three size classes of tree, the DistLM sequential test for the soil bacterial community composition showed that this community was shaped by % moisture and was the best predictor variable in structuring the soil bacterial community composition (Pseudo-F = 3.0378, $p = 0.0012$, AICc = 97.22) (Table 3) and explained 15.96% of the total variation observed in the soil bacterial community composition across the tree sizes in the primary forest (Fig. S3). The soil fungal community composition was shaped by *p*H but this was only marginally significant, (Pseudo-F = 2.4506, $p = 0.0578$, AICc = 125.29) and explained 13.28% of the total variation observed in the soil fungal community composition across the tree sizes in the primary forest (Table 3) (Fig. S4).

In the secondary forest, soil NH_4^+ and C_{mic} together accounted for 40% of the total variation observed in the soil bacterial community composition across the tree sizes (Pseudo-F = 6.5439, $p = 0.0027$, AICc = 121.11 and Pseudo-F = 2.7334, $p = 0.0337$, AICc = 121.01, respectively) (Table 3) (Fig. 2a). Similarly, the DistLM sequential tests for the soil fungal community found that NO_3^- was the best variable (Pseudo-F = 4.0122, $p = 0.0044$, AICc = 127.18) (Table 3) and this explained 20.05% of the total variation observed in the soil fungal community composition across large, medium, and small tree sizes in the secondary forest (Fig. 2b).

Primary vs secondary forest: Pentaclethra large, medium, and small tree-soils

For the soil bacterial and fungal community compositions of *Pentaclethra* large tree-soils between the

Table 1 Analysis of the dissimilarity in the soil bacterial and fungal community composition across *Pentaclethra macroloba* tree size within the primary forest and within the secondary forest in the MNWR

Site	Soil Community	Tree Size Pairwise Comparison	Pseudo-F	*p* value	% Dissimilarity	Cohen's *d*
Primary Forest	Bacteria	Large, Medium	1.2542	0.1317	17.6	0.5279
		Medium, Small	3.9701	0.0023	23.1	0.9393
		Large, Small	3.2278	0.002	22.9	0.8469
	Fungi	Large, Medium	1.9707	0.0159	31.5	0.6618
		Medium, Small	5.2592	0.0035	51.6	1.0811
		Large, Small	4.0485	0.0015	49.0	0.9485
Secondary Forest	Bacteria	Large, Medium	3.2642	0.0482	35.6	0.8517
		Medium, Small	2.5367	0.035	43.7	0.7508
		Large, Small	4.5250	0.012	49.2	1.0028
	Fungi	Large, Medium	2.1266	0.0412	40.8	0.6874
		Medium, Small	3.9665	0.0067	53.7	0.9389
		Large, Small	5.5937	0.0048	56.7	1.1149

The eDNA sequence relative proportion data were analyzed using the multivariate and permutation-based PERMANOVA. The percent dissimilarity, Pseudo-F value, *p* value, and Cohen's *d* effect size are presented in the table

Table 2 Analysis of the dissimilarity in the soil bacterial and fungal community composition of each *Pentaclethra macroloba* tree size classes between the primary forest and the secondary forest in the MNWR

Soil Community	Tree Size	Site Pairwise Comparison	Pseudo-F	*p* value	% Dissimilarity	Cohen's *d*
Bacteria	Large	Primary, Secondary	1.3361	0.1059	24.9	0.5449
	Medium	Primary, Secondary	6.6039	0.0023	33.1	1.2114
	Small	Primary, Secondary	5.5540	0.0157	48.4	1.111
Fungi	Large	Primary, Secondary	1.6579	0.0019	34.9	0.607
	Medium	Primary, Secondary	4.0618	0.0035	41.6	0.9501
	Small	Primary, Secondary	3.9038	0.0022	60.2	0.9314

The eDNA sequence relative proportion data were analyzed using the multivariate and permutation-based PERMANOVA. The percent dissimilarity, Pseudo-F value, *p* value, and Cohen's *d* effect size are presented in the table

primary and secondary forest, no verified 'best solution' could be obtained from the DistLM sequential test (Table 4). However, the soil C:N ratio was the best predictor variable responsible for the soil bacterial

Table 3 The Distance-based linear modeling (DistLM) marginal and sequential tests describing the relationship of the soil abiotic variables and the patterns in the soil bacterial and fungal community composition across large, medium, and small *Pentaclethra macroloba* tree-soils, within the primary forest (left), and within the secondary forest (right) in the MNWR

Primary Forest: Large vs Medium vs Small					Secondary Forest: Large vs Medium vs Small				
Marginal:	SS(trace)	Pseudo-F	*p* value	Prop.	Marginal:	SS(trace)	Pseudo-F	*p* value	Prop.
Soil Bacterial Community Composition					Soil Bacterial Community Composition				
C	193.83	0.9007	0.5402	0.05329	C	538.01	0.5485	0.7287	0.03314
N	133.7	0.6106	0.9297	0.03676	N	450.86	0.45711	0.8219	0.02777
C:N ratio	432.76	2.161	0.0106	0.11899	C:N ratio	805.29	0.83521	0.475	0.04961
NH_4^+	137.72	0.6297	0.918	0.03786	NH_4^+	4711.7	6.5439	0.0024	0.29027
NO_3^-	188.42	0.8741	0.5853	0.05180	NO_3^-	1389.4	1.4978	0.1891	0.08559
C_{mic}	243.9	1.1501	0.2567	0.06706	C_{mic}	1650.8	1.8114	0.1174	0.1017
pH	571.2	2.981	0.0006	0.15705	pH	1590.4	1.738	0.1398	0.09798
Moisture	580.34	3.0378	0.0016	0.15957	Moisture	1297.4	1.39	0.2223	0.07992
Sequential:	AICc	Pseudo-F	*p* value	R^2	Sequential:	AICc	Pseudo-F	*p* value	R^2
Moisture	97.22	3.0378	0.0012	0.15957	NH_4^+	121.11	6.5439	0.0027	0.29027
					C_{mic}	121.01	2.7334	0.0337	0.10939
Soil Fungal Community Composition					Soil Fungal Community Composition				
C	1178.5	1.21	0.2559	0.0703	C	1407	1.1988	0.2569	0.06970
N	948.82	0.9600	0.3447	0.0566	N	1180.3	0.99355	0.3717	0.05846
C:N ratio	1150.8	1.1795	0.2618	0.0686	C:N ratio	1522.4	1.3051	0.2089	0.07541
NH_4^+	566.05	0.5592	0.7408	0.0337	NH_4^+	3283.9	3.1085	0.0085	0.16268
NO_3^-	894.83	0.9023	0.3958	0.0533	NO_3^-	4047.2	4.0122	0.0029	0.20049
C_{mic}	655.13	0.6507	0.6208	0.0390	C_{mic}	1507.7	1.2914	0.2134	0.07468
pH	2226.3	2.4506	0.0542	0.1328	pH	1590	1.368	0.1864	0.07876
Moisture	1401	1.4593	0.1648	0.0835	Moisture	1312.8	1.1129	0.2867	0.06503
Sequential:	AICc	Pseudo-F	*p* value	R^2	Sequential:	AICc	Pseudo-F	*p* value	R^2
pH	125.29	2.4506	0.0578	0.13282	NO_3^-	127.18	4.0122	0.0044	0.20049

The DistLM was performed using stepwise sequential tests following AICc selection criterion. Significant results are indicated by $p < 0.05$ (Prop. var. = proportion of variation out of total variation)

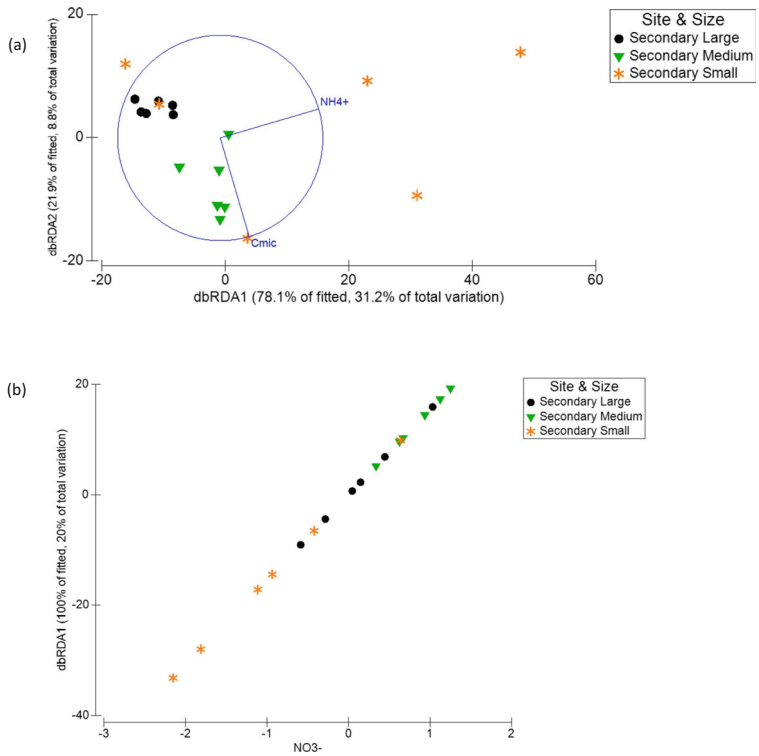

Fig. 2 The Distance-based redundancy analysis (dbRDA) ordination plot of the DistLM sequential test results based on the soil abiotic variables fitted to the variation in the **a** soil bacterial and **b** fungal community composition between large, medium, and small *Pentaclethra macroloba* tree-soils within the secondary forest. Soil NH_4^+ and C_{mic} were the best predictor variables out of the model, and together, these soil abiotic variables explained 40% of the total variation observed in the **a** soil bacterial community patterns across the three *P. macroloba* tree sizes in the secondary forest (NH_4^+: Pseudo-F = 6.54, $p = 0.0027$, AICc = 121.11, and C_{mic}: Pseudo-F = 2.73, $p = 0.034$, AICc = 121.01) (Table 3). Soil NO_3^- was the best predictor variable out of the model and explained 20% of the total variation observed in the **b** soil fungal community patterns across the three *P. macroloba* tree sizes within the secondary forest (Pseudo-F = 4.01, $p = 0.0044$, AICc = 127.18) (Table 3)

community composition variation between primary forest medium tree-soils and the secondary forest medium tree-soils (Pseudo-F = 3.4166, $p = 0.0185$, AICc = 73.985) and explained 25.47% of the total variation observed in the soil bacterial community composition (Table 4) (Fig. S5). Whereas, the soil bacterial DistLM sequential tests found that NH_4^+ and NO_3^- together best explained the soil bacterial community composition of the small tree-soils between the primary forest and the secondary forest (Pseudo-F = 6.7427, $p = 0.0035$, AICc = 81.536 and Pseudo-F = 4.8125, $p = 0.0061$, AICc = 80.062, respectively) (Table 4) and these two variables accounted for 61.08% of the total variation in these soil bacterial communities (Fig. 3a). Similarly, the soil fungal community composition, soil NO_3^- was the best predictor variable from the results of the DistLM sequential test (Pseudo-F = 3.8259, $p = 0.0017$, AICc = 88.716) (Table 4) and this explained 27.67% of the total variation observed in the soil fungal community composition of the small tree-soils between the primary and secondary forest (Fig. 3b).

Discussion

In this study, we assessed the soil bacterial and fungal community composition and their determinants associated with large- ($\geq$ 30 cm DBH), medium- (5 cm $\leq$ DBH $\leq$ 10 cm), and small- ($\leq$ 1 cm DBH) sized *Pentaclethra* trees in both a primary forest and a 23-year-old secondary forest of the same soil texture and topography, but managed differently in the past 23 years. Given the large amount of comparisons and results presented, here, we focus our discussion on the main findings of this study. The results of this study provides evidence that as *Pentaclethra* gets larger in size (or gets

Table 4 The Distance-based linear modeling (DistLM) marginal and sequential tests describing the relationship of the soil abiotic variables and the patterns in the soil bacterial and fungal community composition of medium (left) and small (right) *Pentaclethra macroloba* tree-soils between the primary forest and the secondary forest in the MNWR. The DistLM was performed using stepwise sequential tests following AICc selection criterion. Significant results are indicated by $p < 0.05$ (Prop. var. = proportion of variation out of total variation). There were no significant DistLM results observed between the large *P. macroloba* tree-soils in the primary forest and large *P. macroloba* tree-soils in the secondary forest.

Large Trees: Primary Forest vs Secondary Forest					Medium Trees: Primary Forest vs Secondary Forest					Small Trees: Primary Forest vs Secondary Forest				
Marginal:	SS(trace)	Pseudo-F	*p* value	Prop.	Marginal:	SS(trace)	Pseudo-F	*p* value	Prop.	Marginal:	SS(trace)	Pseudo-F	*p* value	Prop.
Soil Bacterial Community Composition					Soil Bacterial Community Composition					Soil Bacterial Community Composition				
C	307.01	0.903	0.4944	0.08285	C	274.31	0.59123	0.7125	0.05582	C	1254.7	1.2241	0.2804	0.10906
N	259.24	0.75225	0.5607	0.06996	N	343.07	0.75057	0.5609	0.06981	N	1150.7	1.1114	0.3235	0.10002
C:N ratio	345.83	1.0294	0.4239	0.09333	C:N ratio	1251.3	3.4166	0.019	0.25465	C:N ratio	1028.9	0.98223	0.3882	0.08943
NH_4^+	125.16	0.3496	0.9818	0.03377	NH_4^+	317.63	0.69106	0.6257	0.06463	NH_4^+	4633.2	6.7427	0.0032	0.40273
NO_3^-	273.96	0.79838	0.5062	0.07393	NO_3^-	558.72	1.2829	0.2439	0.1137	NO_3^-	3945.1	5.2189	0.0039	0.34292
C_{mic}	309.78	0.91231	0.4357	0.08360	C_{mic}	590.63	1.3662	0.2412	0.1202	C_{mic}	1376.3	1.3588	0.2315	0.11963
*p*H	377.67	1.1349	0.2486	0.10193	*p*H	858.57	2.1171	0.077	0.17472	*p*H	2930	3.4172	0.026	0.25469
Moisture	586.78	1.8816	0.0845	0.15836	Moisture	692.28	1.6398	0.1575	0.14088	Moisture	1881.1	1.9547	0.125	0.16351
Sequential:	AICc	Pseudo-F	*p* value	R^2	Sequential:	AICc	Pseudo-F	*p* value	R^2	Sequential:	AICc	Pseudo-F	*p* value	R^2
+Moisture	72.056	1.8816	0.0855	0.15836	C:N ratio	73.985	3.4166	0.0185	0.25465	NH_4^+	81.536	6.7427	0.0035	0.40273
-Moisture	71.192	1.8816	0.0924	0.15836						NO_3^-	80.062	4.8125	0.0061	0.2081
Soil Fungal Community Composition					Soil Fungal Community Composition					Soil Fungal Community Composition				
C	684.19	1.1613	0.2422	0.10404	C	562.67	0.73101	0.6924	0.068122	C	2231.7	1.4828	0.1977	0.12913
N	606.93	1.0168	0.4245	0.09229	N	597.2	0.77937	0.6469	0.072302	N	2173.1	1.4382	0.2082	0.12574
C/N	673.87	1.1418	0.2704	0.10248	C:N ratio	1758.2	2.7042	0.0135	0.21286	C:N ratio	1870.2	1.2134	0.3015	0.10821
NH_4^+	404.1	0.65474	0.9246	0.06145	NH_4^+	735.04	0.97682	0.4021	0.08899	NH_4^+	2612.2	1.7806	0.1133	0.15115
NO_3^-	586.29	0.97884	0.4805	0.08915	NO_3^-	781.4	1.0449	0.3401	0.094603	NO_3^-	4782.4	3.8259	0.0019	0.27672
C_{mic}	597.55	0.99951	0.4298	0.09086	C_{mic}	1064.1	1.4788	0.1448	0.12883	C_{mic}	2017.7	1.3218	0.2435	0.11675
*p*H	585.09	0.97664	0.441	0.08897	*p*H	1090	1.5203	0.122	0.13197	*p*H	2679.4	1.8349	0.104	0.15504
Moisture	670.73	1.1358	0.28	0.10200	Moisture	868.63	1.1752	0.2606	0.10516	Moisture	1838.4	1.1903	0.3083	0.10637
Sequential:	AICc	Pseudo-F	*p* value	R^2	Sequential:	AICc	Pseudo-F	*p* value	R^2	Sequential:	AICc	Pseudo-F	*p* value	R^2
+C	79.69	1.1613	0.2455	0.10404	+C:N ratio	80.872	2.7042	0.015	0.21286	NO_3^-	88.716	3.8259	0.0017	0.27672
-C	78.075	1.1613	0.241	0.10404	-C:N ratio	80.811	2.7042	0.0128	0.21286					

No best solution

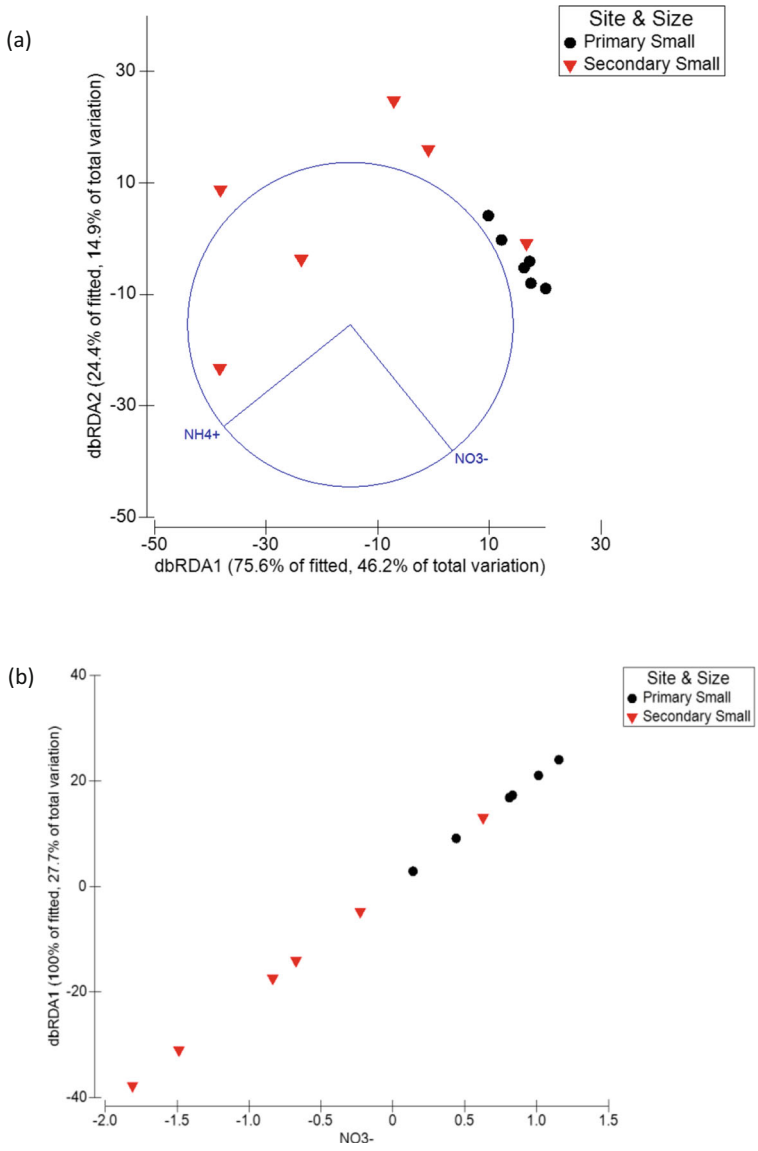

Fig. 3 The Distance-based redundancy analysis (dbRDA) ordination plot of the DistLM sequential test results based on the soil abiotic variables fitted to the variation in the **a** soil bacterial and **b** fungal community of *Pentaclethra macroloba* small tree soils between the primary forest and the secondary forest. Soil NH_4^+ and NO_3^- were the best predictor variable out of the model, and together, explained 61.08% of the total variation observed in the **a** soil bacterial community patterns between the primary forest *P. macroloba* small tree-soils and the secondary forest *P. macroloba* small tree-soils (NH_4^+: Pseudo-F = 6.74, $p = 0.0035$, AICc = 81.54, and NO_3^-: Pseudo-F = 4.01, $p = 0.0061$, AICc = 80.06) (Table 4). Soil NO_3^- was the best predictor variable out of the model and explained 27.7% of the total variation observed in the **b** soil fungal community patterns between the primary forest *P. macroloba* small tree-soils and the secondary forest *P. macroloba* small tree-soils (Pseudo-F = 3.83, $p = 0.0017$, AICc = 88.72) (Table 4)

older in age) this may influence its affiliated soil microbiome, and that even if an early community is initially very dissimilar in contrasting land-use histories (i.e. primary forest or secondary forest) can become increasingly similar over a tree developmental stage (Fig. 4). In other words, soil legacy effects from land-use history may be mediated in plant-soil microbiomes through tree size (or age) of a plant species. Therefore, a plant-species may be able to mediate soil microbial succession through plant 'Zinke' effects and their affiliated soil microbial biome if they are capable of creating similarity in soil microbial community composition over a plant developmental period, even in contrasting land-use histories. Moreover, we provide evidence the important role that the initial or early starting conditions of soil, pertaining to soil N, may play a relatively large role in which soil microbial groups come together to form the early plant-soil microbial community affiliated with *Pentaclethra* that could have overall implications for structuring soil microbial communities in contrasting land-use histories.

For the most part, the soil bacterial and fungal community composition was different across *Pentaclethra* tree size within the primary forest, and within the secondary forest. Indeed, many biological processes have been shown to change in rate, as organisms grow and

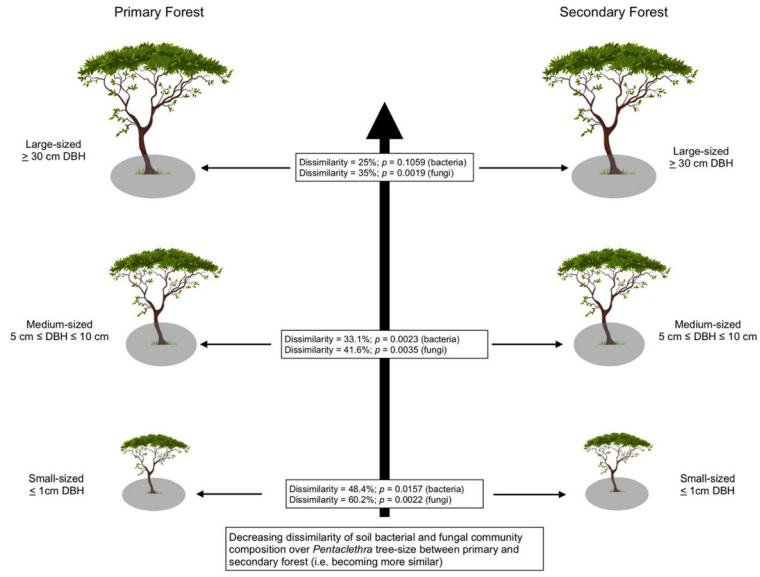

Fig. 4 Schematic of the decreasing dissimilarity of the soil bacterial and fungal community composition affiliated with the three *Pentaclethra* tree-sizes between a primary and secondary forest

age (e.g. Haldane 1926; Schmidt-Nielsen 1975)—almost all organisms on earth are multicellular and exhibit ontogenetic growth from birth to death (Wilbur 1980). As tree size increases, there are associated increases in the biomechanical loading of not only stem tissues (Thomas and Winner 2002), but also carbon costs that have been used to predict maximal tree height (Givnish 1988). As trees of different sizes and heights have varying access to light through canopy gaps and vertical stratification, this would potentially influence the amount of fixed C entering the soil through root exudates that is available to the surrounding soil. Therefore, as *Pentaclethra* increases in size (or ages), its resource requirement would presumably change as well, or that canopy gap dynamics could have potential consequences of the amount of photosynthate entering the adjacent soil environment.

Our findings suggest that as trees develop from the smaller size stages to the medium size trees, there is a significant change in the bacterial community composition, and as the trees age from small, medium, to large, there is a consistent shift in the fungal community structure. This may be reflecting the size of the tree, or some change in root exudate that occurs with tree-age. Interestingly, over a tree developmental period, it is clear that the soil bacterial communities can become more similar in primary forest soil conditions. This may be due to the simpler metabolism and shorter generation time of bacteria as compared to fungi, the greater ability of bacteria to share metabolic genes horizontally in concert with the greater functional redundancy typically found in the bacterial population (Allison et al. 2008; Allison and Martiny 2008). In contrast, fungal populations commonly take more time to develop, as they are more resource and niche based with a more fastidious metabolism than soil bacteria, and are more dependent on changes in the environment during soil succession. All of these could result in the soil bacterial populations becoming more similar over a shorter duration, than the soil fungal community does in the primary forest. Therefore, during early development of a tree soil microbiome in the primary forest, the microbial communities are in a state of high species turnover as they establish dominance, resulting in more taxonomic fluctuations occurring in soil microbiome community structure, which appears to become more stable over time (Brussaard 1997; Griffiths et al. 2000; Brussaard et al. 2007). Moreover, soil conditions may also influence the soil microbiome associated with different tree sizes. For example, the soil bacterial community composition across tree-size in the primary forest, was structured by % moisture (~16%). Whereas in the secondary forest, soil NH_4^+ and C_{mic} was responsible for 40% of the total variation observed in the soil bacterial community composition across tree-size.

Previous evidence has shown that rhizosphere bacterial and fungal communities to change according to a plant developmental gradient (Chaparro et al. 2012), and a more recent study has shown that microbial communities to be distinct at the seedling stage from other

plant developmental time periods (Chaparro et al. 2014). However, the rhizosphere is a very distinct ecotype that is different than the overall bulk-soil ecosystem adjacent to the tree. In addition, many of these studies whether rhizosphere changes over plant development or 'Zinke' effects, neither have examined these processes in contrasting land-use histories, to understand how microbial communities may change or become more similar, even when developed in different starting soil conditions. It has been suggested that legacy effects of plant-soil microbiomes can be mediated by ontogenetic niche shifts of a plant (Wurst and Ohgushi 2015), yet, it is currently not fully understood the details of community consequences of individual-level ontogenetic processes (Nakazawa 2014).

The percent dissimilarity of the bacterial community across tree size between primary and secondary forest decreased (i.e., community composition becoming more similar) moving from small to large trees, such that there was no significant difference in bacterial composition of large trees between the primary forest and secondary forest. This indicates that even if the starting soil microbiome is initially very dissimilar between contrasting land-use histories, certain tree species could lead to soil microbial community composition becoming similar (as indicated by a decreasing trend in percent dissimilarity difference in the soil bacterial community composition across tree size between the primary forest and the secondary forest). Therefore, these soil bacterial communities can become more similar over tree-size or as a tree ages even in contrasting land-use histories. This indicates legacy effects may be mediated by tree size and age or ontogenetic niche shifts, as has been described elsewhere (Wurst and Ohgushi 2015). Ontogenetic niche shifts by *Pentaclethra* may mediate plant 'Zinke' effects on the soil microbial community structure and dynamics (Nakazawa 2010, 2014) and different soil conditions, indicating importance for legacy effects. Thus, both the fungal and bacterial communities show a trend towards decreased dissimilarity over tree developmental life stages, even in contrasting land-use histories of soil (e.g. Fig. 4).

However, the soil fungal community composition was significantly different between primary and secondary forest large-tree-soils. Given this, it may be that the soil fungal community composition will require more time to become more homogenized. This suggests that smaller trees may have more ecosystem heterogeneity associated with their soils, as they are still in a highly variable stage of development, and these soil microbial communities may be more susceptible to stochastic processes. This suggests that the younger tree soils or more recently damaged soil habitats have an increase in microbial heterogeneity.

Bacteria were shaped by NH_4^+ and NO_3^-, which explained 61.1% of the total variation, suggesting some strong evidence that forms of inorganic N are important for early development of the bacterial community. Fungal community structure was also shaped by NO_3^-, which explained 27.7% of the total variation. The greater level of NH_4^+ in secondary-small tree soils than in the primary forest, but greater levels of NO_3^- in primary-small tree soils than in the secondary forests follows a typical forest successional pattern associated with a likely greater level of ammonium-oxidation occurring in the primary forest soils. Soil NH_4^+ and NO_3^- explained 61% of the variation in the soil bacterial community composition associated with primary small trees and secondary small trees. Thus, this points that either the previous land use activity has created overall changes in the soil abiotic conditions that is then mediated through plant growth, or that the plant produces different substrates in the soil based on prior land use history. Moreover, even if NH_4^+ and NO_3^- are responsible for 61% of the variation observed between small primary and small secondary, the *Pentaclethra* plant itself may help to lead to community similarity over a plant developmental period.

From the overall results of this study, it is clear that not only does the developmental stage of this N-fixing plant species have an influence on its soil microbiome, but also, previous land-use history of soil can have an influence as well, as a result of possible changes in light and soil conditions. As this plant species develops over time in habitats with contrasting soil histories, the soil bacterial community composition can become more similar, as was observed in this study, however, the fungal community may take more time, but still follow the increasing similarity pattern. The results of the current study support the concept that as *Pentaclethra* ages and develops in soils of contrasting past land-use histories, the soil microbiome community composition can become similar over time. Yet, even though the small trees in this study have initially colonized in soils with contrasting land-use histories, and had different initial soil bacterial and fungal community compositions between the primary forest and the secondary forest, there is evidence to suggest, that over a developmental time

period, these communities can become more similar, even though these different developmental stages of trees have different starting soil abiotic conditions. In addition, it is clear that as a tree develops in its respective area, its associated soil microbiome will also shift, suggesting that these two components go through succession or developmental stages together. What is unclear is which comes first, and what this means (meaning is there a lag response in the soil microbial community development, as they catch up with the ecophysiological and metabolic needs of the plant as it ages/develops). This could have important implications for understanding changes or shifts in soil microbial community dynamics as a result of plant tree-size/developmental stage that can help guide forest restoration and warrants more investigation in this region.

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Compliance with ethical standards

Competing interests All authors declare no competing interests.

References

Abrams MD, Hock WK (2006) Annual growth rings and the impact of Benlate 50 DF fungicide on citrus trees in seasonally dry tropical plantations of northern Costa Rica. For Ecol Manag 227:96–101. https://doi.org/10.1016/j.foreco.2006.02.019

Akaike H (1978) A new look at the Bayes procedure. Biometrika 65:53. https://doi.org/10.2307/2335276

Allison SD, Martiny JBH (2008) Colloquium paper: resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci U S A 105(Suppl 1):11512–11519. https://doi.org/10.1073/pnas.0801925105

Allison SD, Czimczik CI, Treseder KK (2008) Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Glob Chang Biol 14:1156–1168. https://doi.org/10.1111/j.1365-2486.2008.01549.x

Anderson MJ, Willis TJ (2003) Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84:511–525. https://doi.org/10.1890/0012-9658(2003)084[0511:CAOPCA]2.0.CO;2/full

Anderson MJ, Gorley RN, Clarke RK (2008) PERMANOVA+ for primer: guide to software and statistical methods. PRIMER-E, Ltd., Plymouth

Barberán A, McGuire KL, Wolf JA et al (2015) Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest. Ecol Lett 18:1397–1405. https://doi.org/10.1111/ele.12536

Bauhus J, Pare D, Cote L (1998) Effects of tree species, stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. Soil Biol Biochem 30:1077–1089. https://doi.org/10.1016/S0038-0717(97)00213-7

Bélanger N, Van Rees C (2006) Sampling forest soils. In: Carter MR, Gregorich EG (eds) Soil sampling and methods of analysis, second edition. Taylor & Francis Group, LLC, Boca Raton, pp 39–46

Binkley D, Giardina C (1998) Why do tree species affect soils? The warp and woof of tree-soil interactions. Biogeochemistry 42:89–106. https://doi.org/10.1023/A:1005948126251

Booth MS, Stark JM, Rastetter E (2005) Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. New Phytol 75:139–157. https://doi.org/10.1890/04-0988

Bruckner A, Barth G, Scheibengraf M (2000) Composite sampling enhances the confidence of soil microarthropod abundance and species richness estimates. Pediobiologia 44:63–74

Brussaard L (1997) Biodiversity and ecosystem functioning in soil. Ambio 26:563–570

Brussaard L, de Ruiter PC, Brown GG (2007) Soil biodiversity for agricultural sustainability. Agric Ecosyst Environ 121:233–244. https://doi.org/10.1016/j.agee.2006.12.013

Buckley DH, Schmidt TM (2003) Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environ Microbiol 5(6):441–452

Bürgi M, Östlund L, Mladenoff DJ (2016) Legacy effects of human land use: ecosystems as time-lagged systems. Ecosystems 20:94–103. https://doi.org/10.1007/s10021-016-0051-6

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci 108:4516–4522. https://doi.org/10.1073/pnas.1000080107

Chaparro JM, Sheflin AM, Manter DK, Vivanco JM (2012) Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils 48:489–499. https://doi.org/10.1007/s00374-012-0691-4

Chaparro JM, Badri DV, Vivanco JM (2014) Rhizosphere microbiome assemblage is affected by plant development. ISME J 8:790–803. https://doi.org/10.1038/ismej.2013.196

Chazdon RL, Broadbent EN, Rozendaal DMA, Bongers F, Zambrano AMA, Aide TM, Balvanera P, Becknell JM, Boukili V, Brancalion PHS, Craven D, Almeida-Cortez JS, Cabral GAL, de Jong B, Denslow JS, Dent DH, DeWalt SJ, Dupuy JM, Durán SM, Espírito-Santo MM, Fandino MC, César RG, Hall JS, Hernández-Stefanoni JL, Jakovac CC, Junqueira AB, Kennard D, Letcher SG, Lohbeck M, Martínez-Ramos M, Massoca P, Meave JA, Mesquita R, Mora F, Muñoz R, Muscarella R, Nunes YRF, Ochoa-

Gaona S, Orihuela-Belmonte E, Peña-Claros M, Pérez-García EA, Piotto D, Powers JS, Rodríguez-Velazquez J, Romero-Pérez IE, Ruíz J, Saldarriaga JG, Sanchez-Azofeifa A, Schwartz NB, Steininger MK, Swenson NG, Uriarte M, van Breugel M, van der Wal H, Veloso MDM, Vester H, Vieira ICG, Bentos TV, Williamson GB, Poorter L (2016) Carbon sequestration potential of second-growth forest regeneration in the Latin American tropics. Sci Adv 2: e1501639–e1501639. https://doi.org/10.1126/sciadv.1501639

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18:117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Clarke KR, Gorley RN (2006) PRIMER v6: user manual/tutorial (Plymouth routines in multivariate ecological research)

DiStefano J, Fidler F, Cumming G (2005) Effect size estimates and confidence intervals: an alternative focus for the presentation and interpretation of ecological data. Nova Science Publishers Inc, New York

Eaton WD, Anderson C, Saunders EF et al (2012) The impact of Pentaclethra macroloba on soil microbial nitrogen fixing communities and nutrients within developing secondary forests in the northern zone of Costa Rica. Trop Ecol 53:207–214

Enquist BJ, Leffler AJ (2001) Long-term tree ring chronologies from sympatric tropical dry-forest trees: individualistic responses to climatic variation. J Trop Ecol 17:41–60. https://doi.org/10.1017/S0266467401001031

Feldpausch TR, Rondon MA, Fernandes ECM, Riha SJ, Wandelli E (2004) Carbon and nutrient accumulation in secondary forests regenerating on pastures in Central Amazonia. Ecol Appl 14:164–176. https://doi.org/10.1890/01-6015

Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology 88:1354–1364. https://doi.org/10.1890/05-1839

Fierer N, Nemergut D, Knight R, Craine JM (2010) Changes through time: integrating microorganisms into the study of succession. Res Microbiol 161:635–642. https://doi.org/10.1016/j.resmic.2010.06.002

Foster D, Swanson F, Aber J et al (2003) The importance of land-use legacies to ecology and conservation. Bioscience 53:77–88

Fukami T, Nakajima M, Fortunel C, Fine PVA, Baraloto C, Russo SE, Peay KG (2017) Geographical variation in community divergence: insights from tropical Forest Monodominance by Ectomycorrhizal trees. Am Nat 190:S105–S122. https://doi.org/10.1086/692439

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Gehring C, Vlek PLG, de Souza LAG, Denich M (2005) Biological nitrogen fixation in secondary regrowth and mature rainforest of Central Amazonia. Agric Ecosyst Environ 111:237–252. https://doi.org/10.1016/j.agee.2005.06.009

Gei MG, Powers JS (2013) Do legumes and non-legumes tree species affect soil properties in unmanaged forests and plantations in Costa Rican dry forests? Soil Biol Biochem 57: 264–272. https://doi.org/10.1016/j.soilbio.2012.09.013

Givnish TJ (1988) Adaptation to sun and shade: a whole-plant perspective. Funct Plant Biol 15:63–92

Gougoulias C, Clark JM, Shaw LJ (2014) The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. J Sci Food Agric 94:2362–2371. https://doi.org/10.1002/jsfa.6577

Griffiths BS, Ritz K, Bardgett RD, Cook R, Christensen S, Ekelund F, Sorensen SJ, Baath E, Bloem J, de Ruiter PC, Dolfing J, Nicolardot B (2000) Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship. Oikos 90:279–294. https://doi.org/10.1034/j.1600-0706.2000.900208.x

Guariguata MR, Ostertag R (2001) Neotropical secondary forest succession: changes in structural and functional characteristics. For Ecol Manag 148:185–206. https://doi.org/10.1016/S0378-1127(00)00535-1

Haldane JBS (1926) On being the right size. phys.ufl.edu

Hart SC, Binkley D, Perry DA (1997) Influence of red alder on soil nitrogen transformations in two conifer forests of contrasting productivity. Soil Biol Biochem 29:1111–1123. https://doi.org/10.1016/S0038-0717(97)00004-7

Hartshorn GS, Hammel B, McDade LA et al (1994) Vegetation types and floristic patterns. La Selva: ecology and natural history of a neotropical rain forest. The University of Chicago Press, Chicago

Hobbie SE (1992) Effects of plant-species on nutrient cycling. Trends Ecol Evol 7:336–339. https://doi.org/10.1016/0169-5347(92)90126-v

Hogan JA, Zimmerman JK, Thompson J, Nytch CJ, Uriarte M (2016) The interaction of land-use legacies and hurricane disturbance in subtropical wet forest: twenty-one years of change. Ecosphere 7:e01405–e01418. https://doi.org/10.1002/ecs2.1405

Holl KD (1999) Factors limiting tropical rain Forest regeneration in abandoned pasture: seed rain, seed germination, microclimate, and soil. Biotropica 31:229–242. https://doi.org/10.1111/j.1744-7429.1999.tb00135.x

Kardol P, Wardle DA (2010) How understanding aboveground-belowground linkages can assist restoration ecology. Trends Ecol Evol 25:670–679. https://doi.org/10.1016/j.tree.2010.09.001

Kardol P, Souza L, Classen AT (2013) Resource availability mediates the importance of priority effects in plant community assembly and ecosystem function. Oikos 122:84–94. https://doi.org/10.1111/j.1600-0706.2012.20546.x

Kashian DM, Romme WH, Tinker DB, Turner MG, Ryan MG (2013) Postfire changes in forest carbon storage over a 300-year chronosequence of Pinus contorta-dominated forests. New Phytol 83:49–66. https://doi.org/10.1890/11-1454.1

Kimmins JP, Comeau PG, Kurz W (1990) Modeling the interactions between moisture and nutrients in the control of Forest growth. For Ecol Manag 30:361–379

Kuusipalo J, Ådjers G, Jafarsidik Y, Otsamo A, Tuomela K, Vuokko R (1995) Restoration of natural vegetation in degradedImperata cylindricagrassland: understorey development in forest plantations. New Phytol 6:205–210. https://doi.org/10.2307/3236215

Lakshmanan V, Selvaraj G, Bais HP (2014) Functional soil microbiome: belowground solutions to an aboveground problem. Plant Physiol 166:689–700. https://doi.org/10.1104/pp.114.245811

Legendre P, Anderson MJ (1999) Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. New Phytol 69:1–24. https://doi.org/10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2

Legendre P, Borcard D, Peres-Neto PR (2005) Analyzing beta diversity: partitioning the spatial variation of community composition data. Ecol Monogr 75:435–450. https://doi.org/10.1890/05-0549

Leopold AC, Andrus R, Finkeldey A, Knowles D (2001) Attempting restoration of wet tropical forests in Costa Rica. For Ecol Manag 142:243–249. https://doi.org/10.1016/S0378-1127(00)00354-6

Martiny JBH, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, Morin PJ, Naeem S, Øvreås L, Reysenbach AL, Smith VH, Staley JT (2006) Microbial biogeography: putting microorganisms on the map. Nat Rev Microbiol 4:102–112. https://doi.org/10.1038/nrmicro1341

McGee KM, Eaton WD, Shokralla S, Hajibabaei M (2018) Determinants of soil bacterial and fungal community composition toward carbon-use efficiency across primary and secondary forests in a Costa Rican conservation area. Microb Ecol 10:423–167. https://doi.org/10.1007/s00248-018-1206-0

McGee KM, Eaton WD, Porter TM et al (2019) Soil microbiomes associated with two dominant Costa Rican tree species, and implications for remediation_ a case study from a Costa Rican conservation area. Appl Soil Ecol 137:139–153. https://doi.org/10.1016/j.apsoil.2019.02.007

McGuire KL, Zak DR, Edwards IP et al (2010) Slowed decomposition is biotically mediated in an ectomycorrhizal, tropical rain forest. Oecologia 164:785–795. https://doi.org/10.1007/s00442-010-1686-1

McNulty C, Barry D (2009) Tropical rainforest stand dynamics, species composition, growth, and mortality at Laguna del Lagarto, Costa Rica. Mesoamericana 13:23–33

Miki T, Ushio M, Fukui S, Kondoh M (2010) Functional diversity of microbial decomposers facilitates plant coexistence in a plant-microbe-soil feedback model. Proc Natl Acad Sci U S A 107:14251–14256. https://doi.org/10.1073/pnas.0914281107

Nakazawa T (2010) Ontogenetic niche shift, food-web coupling, and alternative stable states. Theor Ecol 4:479–494. https://doi.org/10.1007/s12080-010-0090-0

Nakazawa T (2014) Ontogenetic niche shifts matter in community ecology: a review and future perspectives. Popul Ecol 57:347–354. https://doi.org/10.1007/s10144-014-0448-z

Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, Knelman JE, Darcy JL, Lynch RC, Wickey P, Ferrenberg S (2013) Patterns and processes of microbial community assembly. Microbiol Mol Biol Rev 77:342–356. https://doi.org/10.1128/MMBR.00051-12

Nichols JD, Carpenter FL (2006) Interplanting Inga edulis yields nitrogen benefits to Terminalia amazonia. For Ecol Manag 233:344–351. https://doi.org/10.1016/j.foreco.2006.05.031

Norden N, Angarita HA, Bongers F, Martínez-Ramos M, Granzow-de la Cerda I, van Breugel M, Lebrija-Trejos E, Meave JA, Vandermeer J, Williamson GB, Finegan B, Mesquita R, Chazdon RL (2015) Successional dynamics in Neotropical forests are as uncertain as they are predictable. Proc Natl Acad Sci U S A 112:8013–8018. https://doi.org/10.1073/pnas.1500403112

Pons TL, Perreijn K, Van Kessel C, Werger MJA (2006) Symbiotic nitrogen fixation in a tropical rainforest: 15N natural abundance measurements supported by experimental isotopic enrichment. New Phytol 173:154–167. https://doi.org/10.1111/j.1469-8137.2006.01895.x

Powers JS, Haggar JP, Fisher RF (1997) The effect of overstory composition on understory woody regeneration and species richness in 7-year-old plantations in Costa Rica. For Ecol Manag 99:43–54. https://doi.org/10.1016/S0378-1127(97)00193-X

Powers JS, Montgomery RA, Adair EC, Brearley FQ, DeWalt SJ, Castanho CT, Chave J, Deinert E, Ganzhorn JU, Gilbert ME, González-Iturbe JA, Bunyavejchewin S, Grau HR, Harms KE, Hiremath A, Iriarte-Vivar S, Manzane E, de Oliveira AA, Poorter L, Ramanamanjato JB, Salk C, Varela A, Weiblen GD, Lerdau MT (2009) Decomposition in tropical forests: a pan-tropical study of the effects of litter type, litter placement and mesofaunal exclusion across a precipitation gradient. J Ecol 97:801–811. https://doi.org/10.1111/j.1365-2745.2009.01515.x

Raich JW, Clark DA, Schwendenmann L, Wood TE (2014) Aboveground tree growth varies with belowground carbon allocation in a tropical rainforest environment. PLoS One 9:e100275–e100278. https://doi.org/10.1371/journal.pone.0100275

Richter DD, Markewitz D, Heine PR, Jin V, Raikes J, Tian K, Wells CG (2000) Legacies of agriculture and forest regrowth in the nitrogen of old-field soils. For Ecol Manag 138:233–248. https://doi.org/10.1016/s0378-1127(00)00399-6

Sahrawat KL (2008) Factors affecting nitrification in soils. Commun Soil Sci Plant Anal 39:1436–1446. https://doi.org/10.1080/00103620802004235

Sakamoto Y, Ishiguro M, Kitagawa G (1986) Akaike information criterion statistics. KTK Scientific Publishers, Tokyo

Schmidt-Nielsen K (1975) Scaling in biology: the consequences of size. J Exp Zool 194:287–307. https://doi.org/10.1002/jez.1401940120

Shebitz DJ, Eaton W (2013) Forest structure, nutrients, and pentaclethra macroloba growth after deforestation of Costa Rican lowland forests. ISRN Ecology 2013:1–10. https://doi.org/10.1155/2013/414357

Siddique I, Vieira ICG, Schmidt S, Lamb D, Carvalho CJR, Figueiredo RO, Blomberg S, Davidson EA (2010) Nitrogen and phosphorus additions negatively affect tree species diversity in tropical forest regrowth trajectories. Ecology 91:2121–2131

Sundquist A, Bigdeli S, Jalili R, Druzin ML, Waller S, Pullen KM, el-Sayed YY, Taslimi MM, Batzoglou S, Ronaghi M (2007) Bacterial flora-typing with targeted, chip-based pyrosequencing. BMC Microbiol 7:108–111. https://doi.org/10.1186/1471-2180-7-108

Tedersoo L, Bahram M, Cajthaml T, Põlme S, Hiiesalu I, Anslan S, Harend H, Buegger F, Pritsch K, Koricheva J, Abarenkov K (2016) Tree diversity and species identity effects on soil fungi, protists and animals are context dependent. ISME J 10:346–362. https://doi.org/10.1038/ismej.2015.116

Thomas SC, Winner WE (2002) Photosynthetic differences between saplings and adult trees: an integration of field results by meta-analysis. Tree Physiol 22:117–127

Ushio M, Wagai R, Balser TC, Kitayama K (2008) Variations in the soil microbial community composition of a tropical montane forest ecosystem: does tree species matter? Soil Biol Biochem 40:2699–2702. https://doi.org/10.1016/j.soilbio.2008.06.023

van der Gast CJ, Gosling P, Tiwari B, Bending GD (2011) Spatial scaling of arbuscular mycorrhizal fungal diversity is affected by farming practice: spatial scaling of arbuscular mycorrhizal fungi. Environ Microbiol 13:241–249. https://doi.org/10.1111/j.1462-2920.2010.02326.x

van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

Vitousek PM (1984) Litterfall, nutrient cycling, and nutrient limitation in tropical forests. Ecology 65:285–298. https://doi.org/10.2307/1939481

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13:87–115

Vitousek PM, Sanford RL (1986) Nutrient cycling in moist tropical Forest. Annu Rev Ecol Syst 17:137–167. https://doi.org/10.1146/annurev.es.17.110186.001033

Wardle DA (2002) Communities and ecosystems. Princeton University Press, Princeton

Wardle DA (2006) The influence of biotic interactions on soil biodiversity. Ecol Lett 9:870–886. https://doi.org/10.1111/j.1461-0248.2006.00931.x

Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten W, Wall DH (2004) Ecological linkages between aboveground and belowground biota. Science 304:1629–1633. https://doi.org/10.1126/science.1094875

Waring BG, Álvarez-Cansino L, Barry KE, Becklund KK, Dale S, Gei MG, Keller AB, Lopez OR, Markesteijn L, Mangan S, Riggs CE, Rodríguez-Ronderos ME, Segnitz RM, Schnitzer SA, Powers JS (2015) Pervasive and strong effects of plants on soil chemistry: a meta-analysis of individual plant 'Zinke' effects. Proc R Soc B Biol Sci 282:20151001–20151008. https://doi.org/10.1098/rspb.2015.1001

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego

Whiting D (2013) Colorado master gardener garden notes. www.cmg.colostate.edu

Wilbur HM (1980) Complex life cycles. Annu Rev Ecol Syst 11:67–93. https://doi.org/10.2307/2096903?refreqid=search-gateway:f7944b511cbe106da3b5897af8fad788

Wurst S, Ohgushi T (2015) Do plant- and soil-mediated legacy effects impact future biotic interactions? Funct Ecol 29:1373–1382. https://doi.org/10.1111/1365-2435.12456

Zinke PJ (1962) The pattern of influence of individual Forest trees on soil properties. Ecology 43:130–133. https://doi.org/10.2307/1932049

Ziter C, Graves RA, Turner MG (2017) How do land-use legacies affect ecosystem services in United States cultural landscapes? Landsc Ecol 32:1–14. https://doi.org/10.1007/s10980-017-0545-4

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