Applied Soil Ecology 137 (2019) 139–153

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Soil microbiomes associated with two dominant Costa Rican tree species, and implications for remediation: A case study from a Costa Rican conservation area

Katie M. McGee[a,*], William D. Eaton[b], Teresita M. Porter[a,c], Shadi Shokralla[a], Mehrdad Hajibabaei[a]

[a] Centre for Biodiversity Genomics at Biodiversity Institute of Ontario and Department of Integrative Biology, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada
[b] Department of Biology, Pace University, 1 Pace Plaza, New York, NY 10038, USA
[c] Great Lakes Forestry Centre, Natural Resources Canada, Sault Ste. Marie, Ontario, Canada

ARTICLE INFO

Keywords:
Pentaclethra macroloba
Dipteryx panamensis
16S rRNA
ITS rRNA
Costa Rica
Soil

ABSTRACT

It is now widely accepted that the majority of tropical landscapes are in transition from disturbance to recovery. Remediation efforts are occurring in Central and South America, attempting to recuperate the soils, often using indigenous nitrogen (N)-fixing tree species. Tree species-generated soil microbial heterogeneity might be important in facilitating regeneration of forest vegetation growth and, although some work has identified these efforts may enhance the soil carbon (C), there have been few studies conducted on how these trees are affecting the below-ground soil biological dynamics in these regions. Here, we explored how and to what extent individual plant effects of a native N-fixing and non-N-fixing plant has affected the below-ground soil C and N metrics and soil bacterial and fungal community composition. To begin to address this, we examined if there were differences in various soil abiotic factors (ToC, TN, C:N ratio, C_{mic}, NH_4^+, NO_3^-, *p*H, and % moisture) and in the soil bacterial and fungal community composition associated with soil of a native non-N-fixer, *Dipteryx panamensis* and a native N-fixer, *Pentaclethra macroloba*, and in comparison to the primary forest bulk-soil in which these tree species occur. We found that primary forest-soils had the greatest amounts of soil NH_4^+ and C_{mic}, followed by *Pentaclethra*-soils. *Dipteryx*-soils had the least amount of soil C_{mic} and NH_4^+, but the greatest amounts of soil NO_3^-. The PERMANOVA results indicated that the Bray-Curtis soil bacterial and fungal community compositions were significantly different between N-fixing and non-N-fixing tree-soils, and also to that of the primary forest-soils. Our results also demonstrated that soil NH_4^+ best explained the variation observed in the soil C_{mic} patterns (39.2%), and, both the soil bacterial (22.8%) and fungal community composition (18.1%). Furthermore, we provide evidence that the N-fixer *Pentaclethra*-soils stimulates the production of more soil C_{mic} than the non-N-fixer *Dipteryx*-soils. It is clear that these tree species are important in creating changes in the soil microbial community composition, and that NH_4^+ may be associated with a shift in a functional response that may have implications for CUE and remediation in this region.

1. Introduction

Tropical forest ecosystems are critical global drivers of terrestrial primary productivity and biogeochemical cycling (Jobbágy and Jackson, 2000; Nottingham et al., 2015). Yet, these unique and important habitats have suffered various degrading land-use practices such as the conversion to agriculture and cattle pasture (Asner et al., 2009; Gibbs et al., 2010); the impacts of which have been observed at the global scale (Brienen et al., 2015; Laurance, 2007; Nottingham et al., 2015). As soil microbes are key components in biogeochemical and nutrient cycling processes, it is thought that tree species-generated soil microbial heterogeneity may be an important factor in facilitating forest regeneration, and thus, critical to the recovery of tropical forests following disturbance.

Following forest clearing, N-fixing plants with N-fixing microbial root nodule symbionts are considered to be the principal pathway that disturbed forests recuperate N (Eaton et al., 2012; Shebitz and Eaton, 2013). Indeed, remediation attempts throughout Costa Rica have been

* Corresponding author.
E-mail address: kmcgee@uogueph.ca (K.M. McGee).

https://doi.org/10.1016/j.apsoil.2019.02.007
Received 13 May 2018; Received in revised form 20 November 2018; Accepted 9 February 2019
Available online 20 February 2019
0929-1393/

implemented including the plantings of N-fixing tree species such as, *Inga edulis* and *Erythrina poeppigiana*, for the amelioration of damaged soils (Holl, 1999; Nichols and Carpenter, 2006; Siddique et al., 2008). However, these efforts are typically monitored with focus on survival and growth rates of tree seedlings in their respective area, and not analysis of the soil ecosystems (Calvo-Alvarado et al., 2007; Cusack and Montagnini, 2004; Holl, 1999; Nichols and Carpenter, 2006; Siddique et al., 2008). Consequently, this contributes to major uncertainties regarding the soil microbial and environmental drivers associated with N-fixing plants in forest ecosystems of Costa Rica.

Individual tree species are likely to have a direct effect on the surrounding soil bacterial and fungal communities through various mechanisms including the exudation of ions and organic compounds, and leaching of dissolved organic materials (Ayres et al., 2009; Kardol and Wardle, 2010; Mukhopadhyay and Joy, 2010; Prescott and Grayston, 2013; Wardle, 2006; Wardle et al., 2004; Zinke, 1962). Some work has demonstrated that individual tree species influence soil abiotic properties and soil biotic community composition for temperate tree species (Binkley and Giardina, 1998; Hobbie, 1992; Zinke, 1962), but less for tropical tree species (McGuire et al., 2010; Raich et al., 2014; Ushio et al., 2008). Different plant species differ in their substrate qualities and quantities entering the soil which is available to the surrounding soil microbial communities (Bauhus et al., 1998; Hobbie, 1992; Wardle, 2002). Consequently, the composition of these soil microorganisms may be altered by the differing organic matter inputs from different plant species. These individual plant effects on the belowground components may provide mechanistic links to the unexplained stochasticity in forest recovery following disturbance, and, as such, studying these components and interactions have been identified as key research priorities for understanding anthropogenic influences on forest ecosystems (Allison and Martiny, 2008; Foster et al., 2003; Wardle et al., 2004; Wardle and Jonsson, 2014). Yet, a recent meta-analysis has shown that individual plant effects are likely to be lower in managed ecosystems versus unmanaged ecosystems, suggesting that individual plant effects may be less pronounced in a managed ecosystem (Waring et al., 2015). As land-use effects and soil disturbance regimes are likely to have an influence on individual plant effects (Gei and Powers, 2013; Hobbie, 1992; Holl, 1999; Powers et al., 1997; Waring et al., 2015), this highlights the importance of studying N-fixing and non-N-fixing individual plant effects in an unmanaged forest, such as a primary forest, in Costa Rica.

Nitrogen-fixing plant species can help restore soil fertility by the stimulation of plant growth, as they have the ability to increase the production of a neighboring tree via increased N availability and soil biomass development (Binkley and Giardina, 1998; Binkley and Menyailo, 2005; Hart et al., 1997; Wardle, 2002; Gehring et al., 2005; Guariguata and Ostertag, 2001; Nichols and Carpenter, 2006). Soils of N-fixing trees have been shown to have a lower C/N ratio, which may favor bacterial decomposers as opposed to fungal decomposers (Fierer et al., 2009; Harrison and Bardgett, 2010). However, soil fungi produce enzymes targeting a wider range of substrates which can transform soil inputs into more usable forms, and also immobilize materials, that typically enhances the assimilation efficiency of organic C into the soil biomass (Anderson, 2003; Anderson and Domsch, 2010; Brookes, 1995; Moscatelli et al., 2005; Strickland and Rousk, 2010; Waring et al., 2013). As such, this can have potential consequences for the amount of C stored in biomass and other various forms of organic C, rather than as C loss through respiratory maintenance processes (Manzoni et al., 2012; Sinsabaugh et al., 2016). Soil microbial decomposers with a greater carbon use efficiency (CUE) can convert substrates in the soil more efficiently to new biomass, leading to a reduced amount of respiration per unit of C take-up (Bradford and Crowther, 2013; Manzoni et al., 2012; Sinsabaugh et al., 2016). Thus, soil C can be quickly mineralized and respired back to the atmosphere as CO_2, or stored as microbial biomass in soil. Therefore, the interplay between these soil microbes and N-fixing plant species becomes critical for CUE, and potentially C-sequestration (Bradford and Crowther, 2013; Manzoni et al., 2012). What remains unclear, is how the presumed N-fixing individual plant effects, in comparison to non-N-fixing individual plant effects, shape the soil microbial community patterns that may be indicative of CUE.

Pentaclethra macroloba (Willd.) Ktze (Fabaceae) is a fundamental, dominant early colonizing N-fixing tree, and a dominant later stage forest canopy tree in the Northern Zone of Costa Rica (Eaton et al., 2012; Shebitz and Eaton, 2013). Eaton et al. (2012) provided the first preliminary look at the composition of the soil prokaryotic community associated with this tree in the tropical lowlands of Costa Rica and found that *Frankia*, *Rhizobium*, Archaea, and Type II methanotrophs were present and likely involved in recuperating soil N and enhancing the microbial biomass C via the more efficient use of organic C. As such, *Pentaclethra* is thought to be important in N and C cycle dynamics and biomass enhancement, and thus, forest succession (Hartshorn and Hammel, 1994; Pons et al., 2006). Recent studies conducted in Costa Rica have explored soil bacterial and fungal community dynamics associated with *Pentaclethra* (and other vegetation types) (Kivlin and Hawkes, 2016a, 2016b). However, these observations were made by examining a monoculture of *Pentacelthra*. Monoculture dynamics can often be vastly different to that of a highly mixed-species habitat, such as a primary forest (Ke and Miki, 2015). As a result, it is quite possible that this type of experimental field design can mask the role *Pentaclethra* is playing in primary old-growth forests of Costa Rica. Despite the critical ecosystem roles *Pentaclethra* is thought to play, the belowground abiotic and biotic structure associated with it is poorly characterized at best, and generally under-studied. No study to date has evaluated the soil biotic communities and soil edaphic and macronutrients associated with this N-fixing tree species in a primary forest of Costa Rica using high-throughput DNA sequencing methods. Therefore, examining the soil biotic and abiotic factors associated with *Pentaclethra*-soils in primary forests of Costa Rica warrants investigation. It is our hope that if we can understand how differences within these soil microbial communities and abiotic metrics serve as drivers of N-fixing and non-N-fixing plant effects within an unmanaged forest, it could shed light on what the main drivers might be for CUE and soil remediation strategies within secondary forests in the region.

The overall aim of this study was to characterize various *Pentaclethra*-soil abiotic factors and the soil bacterial and fungal community composition in comparison to that of a native non-N-fixing member of the same family, *Dipteryx panamensis* (Pittier) Record and Mell (Fabaceae); and in comparison, to the primary forest floor bulk soil within a primary old-growth forest in the Northern Zone of Costa Rica. The objectives of this study were to determine the individual plant effects of a native N-fixing tree, in comparison to a non-N-fixing tree in the region. Moreover, we wanted to identify which soil abiotic factors might be structuring these soil bacterial and fungal communities across the tree-species soil and primary forest soils to explore potential drivers of these microbial communities that are greater for CUE. To begin to address this, four questions were asked in this study: (i) How do various soil abiotic factors and soil C_{mic} differ across *Dipteryx*- and *Pentaclethra*-soils, and primary forest bulk soils? (ii) Are there certain soil abiotic factors that are associated with changes in the soil C_{mic} among the tree-soils and primary forest-soils? (iii) Are there different soil bacterial and fungal community compositions between the two tree species in comparison to primary forest bulk soils? (iv) Which soil abiotic variables appear to shape the soil bacterial and fungal communities in *Dipteryx*-soils and *Pentaclethra*-soils? These tree species were chosen for three reasons: first, both are dominant members of the forest canopy in this region; second, both are in the same family (Fabaceae); and third, to provide a comparison of two species of the same family, but one is known to form root nodule N-fixing symbionts (*Pentaclethra*), whereas *Dipteryx* has not been shown to form these symbionts (Allen and Allen, 1981; Halliday, 1984; Montagnini and Sancho, 1994).

We predicted that soil bacterial and fungal communities across *Pentaclethra*-, *Dipteryx*-, and primary forest bulk-soil would be different from one another given the known N-fixing characteristics of

Fig. 1. Map of the San Juan La-Selva Biological Corridor (left) and the Maquenque National Wildlife Refuge (right) (MNWR outlined in black and shaded green) (Cove et al., 2014). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Pentaclethra. We also predicted forms of inorganic N would be associated in shaping of these soil bacterial and fungal community compositions. Furthermore, because of the known roles that N-fixing symbionts and different forms of inorganic N play in plant and microbial growth, we predicted *Pentaclethra* would promote more soil microbial biomass C in its soil, in contrast to *Dipteryx*, and would be similar to that of the primary forest bulk soil.

2. Materials and methods

2.1. Site description

In 2001, the San Juan-La Selva Biological Corridor (SJLBC) (Fig. 1) was created by the Costa Rican Ministry of Environment and Energy to protect 1,204,812 ha of diverse ecosystems for habitat connectivity and biodiversity in the Northern Zone of Costa Rica (Monge et al., 2002), due to legal and illegal extraction-based land management practices since the 1970's. In 2005, within the SJLBC, the Maquenque National Wildlife Refuge (MNWR) (10°27′05.7″N, 84°16′24.32″W) was established by executive decree of the Costa Rican government to protect over 50,000 ha of various ecosystems, such as primary and secondary forests, and wetlands (Fig. 1). The MNWR is the core nucleus of the SJLBC for biodiversity as it contains the highest percentage of forest cover and most valuable habitats for biodiversity in the region (Chassot and Monge, 2012). Mean annual temperature is 27 °C, mean annual rainfall is 4300 mm, and the dominant soil type are oxisols (Hartshorn and Hammel, 1994).

2.2. Soil sample collection

This study was conducted in the humid Atlantic lowland rainforest of the MNWR. All field and soil sample collections were conducted in an upland primary forest within the MNWR, with all of the primary forest upland areas consisting of the same soil type (oxisols), soil typography, and soil texture (McGee et al., 2019). Soil samples were collected from adjacent *Dipteryx* and *Pentaclethra* trees that are commonly found within this primary forest. For a control to compare that tree species soil bacterial and fungal communities are unique from one another, and unique from the primary forest floor soil, these soils were compared to primary forest bulk soil samples collected in the previous year (July 2014) from six, 1000 m^2 plots. Throughout these six 1000 m^2 plots in the primary forest, six individual trees per tree species > 30 cm diameter at breast height (DBH) were used for this study. 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 (Abrams and Hock, 2006; Enquist and Leffler, 2001; Jacoby and D'Arrigo, 1990). Furthermore, we were only concerned with examining larger established trees, and therefore did not collect soils associated with different age classes of the tree species, and only collected tree soils of trees > 30 cm DBH.

Six individual trees per tree species were chosen and soil sampling around the tree depended on the DBH of each tree. The DBH of each individual tree was used to establish a tree protection zone (TPZ) radius. The TPZ radius is relative to the DBH of a tree, and Whiting (2013) describes this TPZ area around the tree as the critical root area with direct influence for the sustainability on tree health and vigor, since not every root is essential for tree health. The drip-line method has been commonly used to assess root influence on soil dwelling communities, however, this method can underestimate the important rooting area for most trees, and therefore this method was avoided (French and Juzwik, 1999; Matheny and Clark, 1998; Perry, 1982; Whiting, 2013). To calculate the TPZ radius, formulas described by Whiting (2013) were applied (Fig. S1) and using a compass, four transects were constructed in each cardinal direction. Along the transects, 10% and 20% zones were determined based on the TPZ radius of the tree (Fig. S1). Sampling transects were established in each cardinal direction to provide uniform sampling around the tree to reduce microsite variability given the heterogeneous properties in tropical soils (van der Gast et al., 2010), and to obtain one representative composite soil sample per individual tree (Bélanger and Van Rees, 2006; Bruckner et al., 2000; Carter and Lowe, 1986; Pennock, 2004; Pennock et al., 2006). The justification for only examining the 10% and 20% zones of the TPZ radius is to eliminate overlap with conspecific adults and to minimize the influence of other neighboring plants and try to capture immediately adjacent tree identity effects and were only interested in the soils immediately adjacent to the tree.

One soil profile core (7.5 cm × 15 cm × 1.25 cm) was collected along the North, South, West, and East transects in the 10% and 20% zones. These soil cores per zone and direction were bulked in sterile Whirl-Pak® (Nasco, Fort Atkinson, WI, USA) bags, resulting in one composite soil sample for each individual tree. Soil cores and collection gloves were sterilized with 70% ethanol between each individual tree. The effect of direction was not examined, thus, sterilizing between directions/transects was not necessary. For homogenization, all soil samples were mixed and passed through a sterilized 4-mm sieve at field moist conditions prior to all downstream analyses.

2.3. Soil abiotic factors

To estimate ToC, TN, NH_4^+, and NO_3^-, 200 g of soil from each of the 18 sieved composite soil samples were delivered to the Center for Tropical Agriculture Research and Education (CATIÉ) Laboratories in Turrialba, Costa Rica. At CATIÉ, ToC levels were analyzed via dry combustion analysis (Anderson and Ingram, 1993), total nitrogen by the Kjeldahl method and, NH_4^+ and NO_3^-, were measured from 2 M KCl extracts using the spectrophotometric methods of Alef and Nannipieri (1995). To estimate levels of microbial biomass C (C_{mic}), SIR was used following the methods of Höper (2006). Substrate-induced respiration for measuring C_{mic} was preferred as it measures the amount of living microbial biomass C. Soil percent moisture and *p*H were measured at each soil core location (2 readings × 8 soil core locations = 16 readings per tree) (Fig. S2) during the time of soil sample collection with a Kelway Soil *p*H and Moisture meter (Kelway Instruments Co., Inc., Wyckoff, NJ, USA). Elevation was measured with a Garmin Rino 650 (Garmin International, Olathe, KS, USA) GPS. All nutrient and microbial activity data presented were adjusted for dry weight of the soil.

2.4. eDNA extraction, PCR, and sequencing

Soil environmental DNA (eDNA) were extracted from each of the 18 composite soil samples using three 0.33 g replicate sub-samples for a total of 1 g for each composite soil sample using the MoBio PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). 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) and 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′-ACTCCTACGGGAGCAGCAG-3′ and 16Sv4R 5′-GGACTACARGGTATCTAAT-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′-CTTGGTCATTTAGAGGAAGTAA-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 10× reaction buffer (200 mM Tris-HCl, 500 mM KCl, pH 8.4), 1 µL 50× $MgCl_2$ (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 for 16S and ITS 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. Successful amplification of PCR products were visualized on 1.5% agarose gels 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 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 were dual indexed and sequenced in several Illumina MiSeq runs using a V3 MiSeq sequencing kit (600 cycles – 300 bp × 2) (FC-131-1002 and MS-102-3003).

2.5. Bioinformatic analyses

The subsequent 16S and ITS Illumina generated sequences were processed using semi-automated pipelines. The 16S Illumina paired-end libraries generated forward (R1) and reverse (R2) reads that did not overlap, and therefore the R1 and R2 reads were analyzed separately. Primer sequences were trimmed using CutAdapt v1.10 (Martin, 2011) specifying a minimum Phred score of 20, a minimum sequence length of 150 bp after trimming, and no > 3 N's allowed. Trimmed reads were further processed using USEARCH v9.1.13 (Edgar, 2010) by dereplicating and then denoising the data using the UNOISE2 pipeline that essentially produces operational taxonomic units (OTUs) with a 100% sequence similarity cutoff but also removes putative chimeric sequences, reads with errors, PhiX contamination, as well as removal of rare OTUs with only one or two reads (singletons and doubletons). Previous work has shown that rare OTUs may be particularly prone to sequence errors and add 'noise' to the dataset (Reeder and Knight, 2009; Tedersoo et al., 2010). The remaining OTUs were taxonomically assigned using the Ribosomal Database Project (RDP) classifier v2.12 (Wang et al., 2007) with the 16S training set v16 using the recommended 50% bootstrap support cutoff value at the genus rank as is recommended for query sequences < 250 bp (Claesson et al., 2009). The ITS Illumina paired-end libraries generated forward and reverse reads that did not overlap, and were also analyzed separately. Primer sequences were trimmed using CutAdapt v1.10 (Martin, 2011) specifying a minimum Phred score of 20 and no > 3 N's allowed. For R1 reads, a minimum sequence length of 200 bp was specified and for R2 reads, a minimum of 150 bp was specified. Trimmed reads were processed using USEARCH v9.1.13 (Edgar, 2010) as described above. Furthermore, the Perl-based ITSx tool was used to extract any remaining SSU/5.8S/LSU sequences from the ITS reads (Bengtsson-Palme et al., 2013). Denoised OTUs were taxonomically assigned using the RDP classifier with the UNITE fungal ITS training set 07-04-2014 using a bootstrap support cutoff of 50% for sequences < 250 bp (Liu et al., 2012). All generated sequencing data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under the BioProject accession number PRJNA471321.

2.6. Soil bacterial and fungal diversity and community composition

All 16S and ITS OTUs were organized taxonomically at the genus rank and converted to mean proportion of sequences per sample. The relative proportion of 16S and ITS OTUs were determined by calculating the total number of sequences from each genus within that subsample, and dividing by the total number of sequences per subsample. To examine the soil bacterial and fungal genus richness and diversity for each sample, Margalef's richness (d = (S-1)/Log(N)) and Shannon Index (*H′*) ($H' = -SUM(P_i*\ln(P_i))$) (P_i is the proportion of the total count (abundance) arising from the *i*th species) were calculated in PRIMER-E v6 (Anderson et al., 2008). Soil bacterial and fungal genus community compositions were transformed using a 4th root transformation to account for dominant taxa as well as rare taxa (Anderson et al., 2008). The 4th root transformed 16S and ITS OTUs were then calculated into a Bray-Curtis dissimilarity matrix in PRIMER-E v6 (Anderson et al., 2008).

2.7. Statistical and multivariate analyses

To address question one, a one-way analysis of variance (ANOVA) was used to determine if the soil abiotic properties were significantly different between primary forest bulk-, *Dipteryx*-, and *Pentaclethra*-soils followed by Tukey's HSD and Dunnett's T3 post-hoc tests in SPSS (v.22, Armonk, NY, USA). Prior to analyses, a Shapiro-Wilk test was performed to determine normality of all the data in SPSS (v.25, Armonk, NY, USA) and all data were $p > 0.05$ suggesting normality.

Table 1
Mean and standard error of the soil nutrient and environmental properties of primary forest bulk-soil, *Dipteryx panamensis*-soil, and *Pentaclethra macroloba*-soil and the one-way ANOVA results evaluating the different soil property elements across these soils. Different letters denote significant pairwise comparisons ($p < 0.05$) based on post-hoc analyses.

	Primary forest	*Dipteryx*	*Pentaclethra*	ANOVA	
				F stat	*p* value
C (%)	5.83 ± 0.21	6.62 ± 0.32	6.50 ± 0.27	2.477	0.118
N (%)	0.47 ± 0.02	0.52 ± 0.02	0.51 ± 0.021	2.257	0.139
C/N	12.45 ± 0.16	12.78 ± 0.25	12.66 ± 0.11	0.820	0.459
C_{mic} (μgC/g)	851.92 ± 94.05^{a}	429.87 ± 50.63^{b}	765.28 ± 95.8^{ac}	7.241	**0.006**
NH_4^+ (μgN/g)	11.86 ± 0.69^{a}	1.08 ± 0.35^{b}	6.41 ± 0.30^{c}	114.07	**< 0.0001**
NO_3^- (μgN/g)	31.55 ± 8.07^{a}	43.38 ± 3.23^{ab}	23.30 ± 1.57^{ac}	14.56	**0.002**
*p*H	5.70 ± 0.03	5.75 ± 0.09	5.77 ± 0.14	0.227	0.802
Moisture (%)	56.72 ± 1.16	57.48 ± 4.09	55.69 + 2.33	0.102	0.903
Elevation (m)	58.75 ± 0.60	51.98 ± 6.49	58.18 ± 0.53	0.990	0.395
Average DBH (cm)	n/a	100.58 ± 0.66	51.33 ± 4.43		

Significant ANOVA results are indicated by $p < 0.05$ in bold.

To address question two, a Principal Coordinates Analysis (PCO) was used to examine the overall patterns of the soil C_{mic} across the primary forest bulk soils, and *Dipteryx*- and *Pentaclethra*-soils in relation to the soil ToC, TN, NH_4^+, NO_3^-, *p*H, and moisture. Pearson correlations were then performed of the individual soil abiotic variables with PCO axes to explore potential soil abiotic factors associated with the soil C_{mic} patterns. To further test these relationships, a multivariate multiple regression was used by implementing a Distance-Based Linear Model (DistLM) approach in PRIMER-E v6 (Anderson et al., 2008), to calculate the contribution of each soil abiotic variable responsible for the distribution of the soil C_{mic} across primary forest bulk-, *Dipteryx*-, and *Pentaclethra*-soils. The DistLM was performed using a 'step-wise' selection procedure and an AICc (Akaike's Information Criterion Corrected) selection criterion with 9999 permutations (Anderson et al., 2008; Burnham and Anderson, 1998). The Distance-Based Linear Modeling sequential tests were considered significant, if $p \leq 0.05$. 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 explains the greatest variation in the data cloud (Legendre and Anderson, 1999). Prior to analysis, the soil C_{mic} data were transformed using a square-root transformation, and calculated into a Euclidean distance resemblance matrix.

To address question three, significant differences in the alpha diversity indices for soil bacterial and fungal genera across primary forest bulk-soil and tree species-soil were determined using a one-way ANOVA followed by Tukey's HSD and Dunnett's T3 post-hoc analyses in SPSS (v.25, Armonk, NY, USA). To determine if there were differences in the Bray-Curtis dissimilarity of the soil bacterial and fungal community composition matrices between the tree species-soils and primary forest bulk-soil, a one-way permutational multivariate analysis of variance (PERMANOVA) with main and pair-wise tests based on unrestricted permutations (9999 permutations) were implemented (Clarke, 1993). The PERMANOVA results of the soil microbial community compositions among tree species-soils and primary forest bulk-soil were considered significant, if $p \leq 0.05$. In addition, a Canonical Analysis of Principal Coordinates (CAP) was used to visualize the distinctness of the soil bacterial and fungal community composition across the primary forest bulk-soil and *Dipteryx*- and *Pentaclethra*-soils based on an a priori allocation success, using the PERMANOVA+ guidelines (Anderson et al., 2008; Anderson and Willis, 2003). Strong differences between primary forest bulk soils, and *Dipteryx*- and *Pentaclethra*-soils are represented by CAP axis 1 and CAP axis 2 squared canonical correlations greater than or equal to 0.7, and moderate differences are represented by squared canonical correlations greater than or equal to 0.5–0.69 (Anderson et al., 2008; 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). Cohen's *d* effect size statistics are considered small if $d = 0.2$, medium if $d = 0.5$–0.7, and large if $d > 0.8$.

To address question four, to identify which soil abiotic variables are structuring the observed multivariate patterns of the soil bacterial and fungal communities across *Pentaclethra*-soil, *Dipteryx*-soil, and primary forest bulk-soil, a multivariate multiple regression was used by implementing the DistLM and dbRDA approaches following the aforementioned procedures. This was based on the same biological fourth-root transformed and resemblance matrices and log (x + 1) transformed environmental data (normalized).

All multivariate analyses were performed in PRIMER-E v6 (Clarke and Gorley, 2006) and its add-on PERMANOVA+ (Anderson et al., 2008). Prior to multivariate analyses, draftsman plots (variable pair-wise scatter plots) were used to determine the homogeneity and multicollinearity of each soil abiotic variable (predictor variables). All soil abiotic variables were transformed using the log (x + 1) transformation to correct for skewness and the data standardized using the 'normalize' parameter in PRIMER-E (Anderson et al., 2008; Clarke and Gorley, 2006).

3. Results

3.1. Differences in soil abiotic factors

The soil C_{mic} was significantly greater in the primary forest bulk- and *Pentaclethra*-soils (851.92 ± 94.1 μgC/g and 765.28 ± 95.8 μgC/g, respectively) than in the *Dipteryx*-soils (429.87 ± 50.63 μgC/g) ($F = 7.24$; $p < 0.05$) (Table 1). Soil NH_4^+ was significantly greatest in the primary forest bulk-soil (11.86 ± 0.69 μgN/g) in comparison to both *Pentaclethra*- and *Dipteryx*-soils (6.41 ± 0.73 μgN/g and 1.08 ± 0.35 μgN/g, respectively) while *Pentaclethra*-soils also had significantly greater amounts of NH_4^+ than *Dipteryx*-soils ($F = 114.07$; $p < 0.0001$) (Tables 1, S1). *Dipteryx*-soils had a significantly greater amount of NO_3^- (43.38 ± 3.23 μgN/g) than *Pentaclethra*-soils (23.30 ± 1.57 μgN/g) ($p < 0.05$), but not the primary forest bulk-soil (31.55 ± 8.07 μgN/g) ($p > 0.05$) (Tables 1, S1). No significant differences were observed for C, N, C:N ratio, *p*H, moisture, and elevation across the primary forest bulk-, *Pentaclethra*-, and *Dipteryx*-soils ($p > 0.05$) (Table 1).

3.2. Drivers of soil microbial biomass C

The PCO axis 1 explained 100% of the variation in the soil C_{mic} and the Pearson correlations with the PCO axes (Fig. S3) showed that soil

Table 2
Distance-based linear modeling (DistLM) marginal and sequential tests describing the association of environmental variables and patterns in (a) soil microbial biomass C, (b) bacterial and (c) fungal community composition across primary forest bulk soil, *Dipteryx panamensis*-associated soil, and *Pentaclethra macroloba*-associated soil in the MNWLR, using stepwise sequential tests following AICc selection criterion. Significant results are indicated by $p < 0.05$ in bold (Prop. var. = proportion of total variation). The marginal tests are used to identify how much each predictor variable explains when taken alone, ignoring all other variables and does not include any corrections for multiple testing. The sequential tests are conditional tests of individual predictor variables done in the order specified, where each test examines whether adding that particular variable contributes significantly to the explained variation to account for covariates.

(a) Soil microbial biomass C

Marginal test	SS(trace)	Pseudo-F	*p*-Value	Prop. var.
C	7.931	0.28248	0.5993	0.017349
N	3.8914	0.13736	0.7212	0.085122
C:N ratio	8.9044	0.31784	0.5894	0.019478
NH_4^+	179.11	10.307	**0.0071**	0.39179
NO_3^-	14.509	0.52444	0.4745	0.031737
*p*H	17.149	0.62361	0.4438	0.037513
Moisture	22.759	0.83828	0.3788	0.049784
Sequential test	AICc	Pseudo-F	*p*-Value	Prop. var.
NH_4^+	54.07	10.307	**0.0073**	0.3918

(b) Soil bacterial community composition

Marginal test	SS(trace)	Pseudo-F	*p*-Value	Prop. var.
C	1235.7	2.7415	**0.0216**	0.14628
N	1178.1	2.5932	**0.0251**	0.13947
C:N ratio	650.59	1.3351	0.2012	0.077019
NH_4^+	1922.8	4.7152	**0.0011**	0.22762
NO_3^-	524.7	1.0597	0.3391	0.06211
C_{mic}	990.94	2.1264	0.056	0.11731
*p*H	435.06	0.8688	0.4926	0.051504
Moisture	498.54	1.0035	0.3787	0.059018
Sequential test	AICc	Pseudo-F	*p*-Value	Prop. var.
NH_4^+	110.87	4.715	**0.0014**	0.22762

(c) Soil fungal community composition

Marginal test	SS(trace)	Pseudo-F	*p*-Value	Prop. var.
C	1423.1	1.3579	0.1527	0.078232
N	1208	1.1381	0.2726	0.066408
C:N ratio	1367.8	1.301	0.176	0.075196
NH_4^+	3294.6	3.5389	**0.0013**	0.18112
NO_3^-	1913.3	1.8807	**0.0405**	0.10518
C_{mic}	1485.1	1.4224	0.1293	0.081644
*p*H	776.48	0.71344	0.7708	0.042686
Moisture	843.5	0.77801	0.6902	0.046371
Sequential test	AICc	Pseudo-F	*p*-Value	Prop. var.
NH_4^+	125.73	3.539	**0.0007**	0.18112

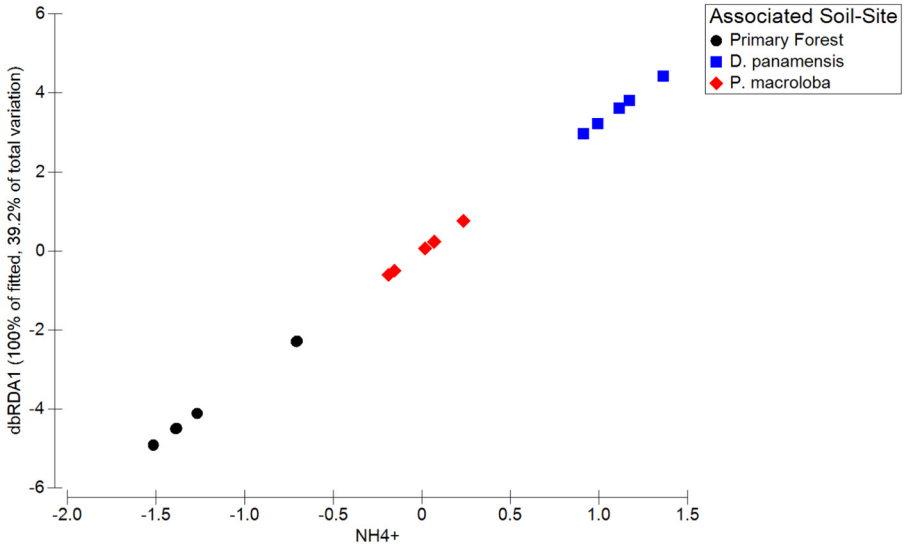

Fig. 2. The distance-based redundancy analysis (dbRDA) ordination plot of the DistLM sequential test results based on the soil abiotic variables fitted to the variation in the soil C_{mic} patterns. Soil NH_4^+ was the best predictor variable out of the model and explained 39.2% of the total variation observed in the soil C_{mic} patterns across the primary, *Dipteryx*-, and *Pentaclethra*-soils (Pseudo-F = 10.31, $p = 0.007$, AICc = 54.07).

Table 3
Analysis of the dissimilarity in overall soil (a) bacterial and (b) fungal community structure across primary forest bulk soil, *Dipteryx panamensis*-soil, and *Pentaclethra macroloba*-soil. The DNA 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.

	Pairwise groups	Pseudo-F	*p*-Value	% dissimilarity	Cohen's *d*
(a) Bacteria	*Dipteryx*, Primary	9.04	0.0022	37.0	1.42
	Dipteryx, *Pentaclethra*	2.79	0.0027	27.8	0.79
	Primary, *Pentaclethra*	10.33	0.0017	36.3	1.52
(b) Fungi	*Dipteryx*, Primary	3.982	0.0023	65.13	0.9497
	Dipteryx, *Pentaclethra*	3.383	0.0028	59.07	0.8671
	Primary, *Pentaclethra*	3.11	0.0026	57.15	0.8313

NH_4^+ was positively moderately correlated with soil C_{mic} ($|r| = 0.626$) across the primary forest bulk-, *Dipteryx*-, and *Pentaclethra*-soils, whereas *p*H was positively weakly correlated to the soil C_{mic} ($|r| = 0.19$) (Table S2). In contrast, C, N, C:N ratio, NO_3^-, and moisture were negatively weakly correlated to the soil C_{mic} ($|r| < 0.25$) (Table S2). The DistLM results indicated that soil NH_4^+ was the best predictor soil variable significantly related to structuring the soil C_{mic} (Pseudo-F = 10.31 $p = 0.007$, AICc = 54.07) (Table 2a) across the associated soils and accounted for 39.18% of the total variation observed in the soil C_{mic} patterns (Fig. 2).

3.3. Differences in the soil bacterial and fungal community composition

The PERMANOVA results showed that the soil bacterial community composition was significantly different for all pairwise comparisons of *Dipteryx*-soil, *Pentaclethra*-soil, and primary forest bulk-soil ($p < 0.05$) (Table 3a). The bacterial communities in the *Dipteryx*-soil and *Pentaclethra*-soil were the least dissimilar in composition at 27.8% (Pseudo-F = 2.79, $p = 0.0027$, $d = 0.79$) (Table 3a), and the primary forest bulk-soil and *Pentaclethra*-soil bacterial communities were dissimilar at 36.3% (Pseudo-F = 10.33, $p = 0.0017$, $d = 1.52$) (Table 3a). The results of the CAP axis 1 squared canonical correlation was 0.9996 indicating a very strong difference in soil bacterial community composition between primary forest bulk-soil, and *Dipteryx*- and *Pentaclethra*-soils and the CAP axis 2 squared canonical correlation was 0.9752, also indicating a strong difference in soil bacterial community composition between *Dipteryx*- and *Pentaclethra*-soils (Fig. 3).

Similarly, the PERMANOVA results showed that the fungal community composition was significantly different for all pairwise comparisons of *Dipteryx*-soil, *Pentaclethra*-soil, and primary forest bulk-soil ($p < 0.05$) (Table 3b). The PERMANOVA results indicated that the soil fungal community composition between *Dipteryx*-soils and primary forest bulk-soil was the most dissimilar (65.13%) (Pseudo-F = 3.982, $p = 0.0023$, $d = 0.9407$) (Table 3b), while the primary forest bulk-soil and *Pentaclethra*-soil were the least dissimilar (57.15%) (Pseudo-F = 3.11, $p = 0.0026$, $d = 0.8313$) (Table 3b). The results of the CAP axis 1 squared canonical correlation was 0.9184, indicating a very strong difference soil fungal community composition between primary forest bulk soil, and *Dipteryx*-soil and *Pentaclethra*-soil (Fig. 5). The CAP axis 2 squared canonical correlation was 0.8178, also indicating strong differences between in the soil fungal community composition between *Dipteryx*-soil and *Pentaclethra*-soil (Fig. 5).

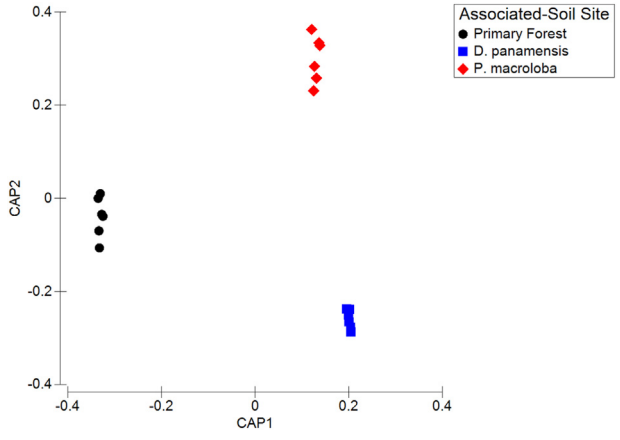

Fig. 3. Canonical Analysis of Principal Coordinates (CAP) showing the strength of the dissimilarity of the overall soil bacterial community composition across the primary forest bulk soil, *Dipteryx*-soil, and *Pentaclethra*-soil in a primary old-growth lowland forest of Costa Rica. The CAP axis 1 squared canonical correlation was 0.9996 and the CAP axis 2 squared canonical correlation was 0.9752.

The primary forest bulk-soil, *Dipteryx*-soil and *Pentaclethra*-soil were mainly dominated by Groups 1, 2, 3, and 5 Acidobacteria, Spartobacteria (genera_incertae_sedis), Subdivision 3 (genera_incertae_sedis), *Nitrospira* (spp.), *Burkholderia* (spp.), *Solibacter* (spp.), and *Bradyrhizobium* (spp.) (Table 4). *Pentaclethra*-soil had the greatest relative percent proportion (RPP) of *Nitrospira* (spp.) (7.3%), followed by *Dipteryx*-soils (3.21%) and least in the primary forest bulk-soil (1.71%) (Table 4). *Dipteryx*-soil had the greatest RPP of N-fixers, represented by *Bradyrhizobium* (spp.), *Rhizomicrobium* (spp.), and *Burkholderia* (spp.) (total RPP of N-fixers (7.54%)), followed by primary forest bulk-soil (6.33%), and least in *Pentaclethra*-soil (4.97%) (Table 4).

The top fungal genera across primary forest bulk-, *Dipteryx*-, and

Table 4
Soil bacterial genus proportion of sequences > 1% representation across primary forest bulk-, *Dipteryx*-, and *Pentaclethra*-soils. 'Groups' are members of Acidobacteria.

Genus	Primary forest	*Dipteryx*	*Pentaclethra*
Gp1	23.57	20.32	18.65
Gp2	24.50	16.54	18.83
Gp3	12.99	18.28	15.90
Spartobacteria Incertae sedis	7.64	6.52	10.16
Subdivision 3 Incertae sedis	4.55	4.82	4.79
Nitrospira	1.71	3.21	7.30
Burkholderia	4.12	3.88	2.83
Solibacter	1.96	3.04	1.67
Gp5	2.17	2.13	1.99
Bradyrhizobium	1.16	2.82	1.44
Edaphobacter	1.20	1.22	0.91
Ktedonobacter	0.42	0.94	1.67
Chitinophaga	1.12	0.10	1.20
Rhizomicrobium	0.97	0.77	0.65
Niastella	0.01	1.13	0.96
Mucilaginibacter	0.26	1.18	0.65
Dyella	1.04	0.33	0.50
Rhodomicrobium	0.30	0.51	0.88
WPS-2 Incertae sedis	0.63	0.59	0.41
Labilithrix	0.20	0.68	0.28
Acidobacterium	0.58	0.41	0.16
Stigmatella	0.00	0.52	0.62
Aggregicoccus	0.52	0.18	0.44
Koribacter	0.78	0.02	0.22
Vampirovibrio	0.10	0.52	0.30
Kitasatospora	0.08	0.68	0.03

Pentaclethra-soils were *Mortierella* (spp.), *Archaeorhizomyces* (spp.), *Trichosporon*, *Geotrichum* (spp.), *Cryptococcus* (spp.), *Myxocephala* (spp.), *Mycena* (spp.) (Table 6). The RPP of *Geotrichum* (spp.) were greatest in *Pentaclethra*-soil (51.69%), whereas primary forest-soil was mainly dominated by *Trichosporon* (spp.) (38.95%), *Mortierella* (spp.) (26.04%), *Cryptococcus* (spp.) (6.17%), *Myxocephala* (spp.) (5.4%), and *Xylaria* (spp.) (4.24%). *Dipteryx*-soil was mostly dominated by *Archaeorhizomyces* (spp.) (24.31%), *Mortierella* (spp.) (21.06%), *Geotrichum* (spp.) (15.95%), *Cryptococcus* (spp.) (15.08%) and *Trichosporon* (spp.) (7.38%). *Pentaclethra*-soil was dominated by *Geotrichum* (spp.) (51.69%), *Trichosporon* (spp.) (18.16%), *Mortierella* (spp.) (15.91%), and *Cryptococcus* (spp.) (2.57%). The primary forest bulk-soil had the greatest RPP of *Trichosporon* (spp.), *Myxocephala* (spp.) and *Xylaria* (spp.), whereas *Dipteryx*-soil had the greatest RPP of *Archaeorhizomyces* (spp.), *Cryptococcus* (spp.), and *Hygrocybe* (spp.), and *Pentaclethra*-soil had the greatest RPP of *Geotrichum* (spp.) (Table 6). *Dipteryx*-soils had the greatest RPP of *Hygrocybe* (spp.) (1.71%), but primary forest bulk-soil and *Pentaclethra*-soils, had the least RPP of *Hygrocybe* (spp.) (0.14% and 0.93%, respectively) (Table 6).

The primary forest bulk soil had significantly greater soil bacterial genus richness than *Dipteryx*- and *Pentaclethra*-soils ($p < 0.0001$), however, no differences were observed in the soil bacterial genus richness between *Dipteryx*-soils and *Pentaclethra*-soils ($p = 0.962$) (Table 5a). Moreover, there were no significant differences in soil bacterial Shannon diversity (H') ($p > 0.05$) (Table 5a).

The primary forest bulk-soil had the greatest soil fungal genus richness in comparison to *Dipteryx*- and *Pentaclethra*-soils ($p < 0.05$), but no differences were observed in fungal genus richness between *Dipteryx*-soil and *Pentaclethra*-soil ($p = 0.948$) (Table 5b). There were no significant differences observed in soil fungal Shannon diversity (H') ($p > 0.05$) (Table 5b).

3.4. Drivers of the soil bacterial and fungal community composition

Soil NH_4^+ was the best predictor variable in structuring the soil bacterial community patterns across the primary forest bulk-, *Pentaclethra*-, and *Dipteryx*-soils (Pseudo-F = 4.72, $p = 0.0019$, AICc = 110.87) (Table 2b) and explained 22.76% of the total variation observed, as visualized by dbRDA (Fig. 4). Similarly, soil NH_4^+ was the best predictor variable in the shaping soil fungal community composition across the primary forest bulk-, *Pentaclethra*-, and *Dipteryx*-soils (Pseudo-F = 3.5389, $p = 0.0007$, AICc = 125.73) (Table 2c) and explained 18.1% of the total variation observed, as visualized by dbRDA (Fig. 6).

4. Discussion

In contrast to the two-way relationship of plant-soil feedbacks, individual plant effects only concern the effects of the tree on the soils beneath and immediately adjacent to the tree (Waring et al., 2015). Individual plant effects are known to influence soil microbial communities (Fierer et al., 2007; Miki et al., 2010; Ushio et al., 2008; Wardle, 2002; Wardle et al., 2004) through the release of biochemicals and dead cells, and by providing photosynthate (Chaparro et al., 2012; Gougoulias et al., 2014; Kimmins et al., 1990; Lakshmanan et al., 2014); the degree of which differs among plant species and successional stage (Bauhus et al., 1998; Wardle, 2006, 2002). Yet, it is unclear what these N-fixing and non-N-fixing individual plant effects may have on the soil bacterial and fungal communities and soil chemistry that may influence potential CUE in unmanaged tropical ecosystems, and the drivers of this. Here, we provide evidence that individual plants effects for N-fixing and non-N-fixing trees have influenced levels of $NH4^+$, NO_3^-, and C_{mic} in the soils. Moreover, we show that NH_4^+ could be the main factor in which to accrue C_{mic} in soils. We also show that the soil bacterial and fungal communities are different, and appear to have influenced the bacterial and fungal groups associated with N-cycling activities. Lastly, we also show that the soil microbial community patterns were driven by NH_4^+. Examining individual plant effects in tropical ecosystems are important in understanding how a tree species may individually affect the soil biotic and abiotic components beneath them. Plant-induced soil microbial heterogeneity will likely have important consequences in the immediately adjacent soils that could have potential consequences for secondary forest developments in the tropics, yet, still warrants more investigation.

Table 6
Soil fungal genera relative percent proportion of sequences > 0.5% representation across the primary forest bulk-, *Dipteryx*-, and *Pentaclethra*-associated soils.

Genus	Primary forest	*Dipteryx*	*Pentaclethra*
Glomeraceae unidentified	0.58	0.76	0.47
Mortierella	26.04	21.06	15.91
Xylaria	4.24	2.17	1.38
Crinipellis	0.29	1.22	0.08
Marasmius	1.07	0.00	0.47
Gymnopus	0.75	0.06	0.00
Hygrocybe	0.14	1.71	0.93
Trichoderma	2.12	0.97	0.20
Viridispora	0.81	0.06	0.00
Pestalotiopsis	0.26	0.14	0.44
Heliscus	0.48	0.13	0.00
Fusarium	1.00	0.00	0.18
Calonectria	1.51	1.01	1.57
Rhizophydium	0.34	0.87	0.19
Synchytrium	0.10	0.83	0.01
Gliocladiopsis	3.04	0.29	0.86
Archaeorhizomyces	1.87	24.31	3.05
Cryptococcus	6.17	15.08	2.57
Mycena	0.26	4.30	1.05
Pseudallescheria	0.83	0.03	0.00
Geotrichum	3.12	15.95	51.69
Trichosporon	38.95	7.38	18.16
Myxocephala	5.40	1.39	0.73

4.1. Differences in soil abiotic factors & determinants of soil C_{mic}

Our results indicated that N-fixing and non-N-fixing individual plant effects can have a substantial influence on plant-essential mineral ions

Table 5
The soil (a) bacterial and (b) fungal genera alpha diversity indices mean ± SE and the one-way ANOVA (n = 6) results followed by post-hoc analyses. The different letters denote significant pairwise comparisons found in the post-hoc analyses ($p < 0.05$).

Community	Diversity index	Primary forest	*Dipteryx*	*Pentaclethra*	ANOVA	
					F stat	*p*-Value
(a) Bacteria	Richness (d)	24.76 ± 0.8^a	17.33 ± 1.56^b	16.94 ± 0.51^{bc}	17.42	< **0.0001**
	Shannon (H')	2.57 ± 0.05	2.72 ± 0.08	2.66 ± 0.03	1.87	0.189
(b) Fungi	Richness (d)	19.94 ± 2.77^a	7.24 ± 0.89^b	7.71 ± 0.44^{bc}	18.03	< **0.0001**
	Shannon (H')	2.25 ± 0.14	2.23 ± 0.09	1.94 ± 0.2	1.48	0.259

Significant ANOVA results are indicated by $p < 0.05$ in bold.

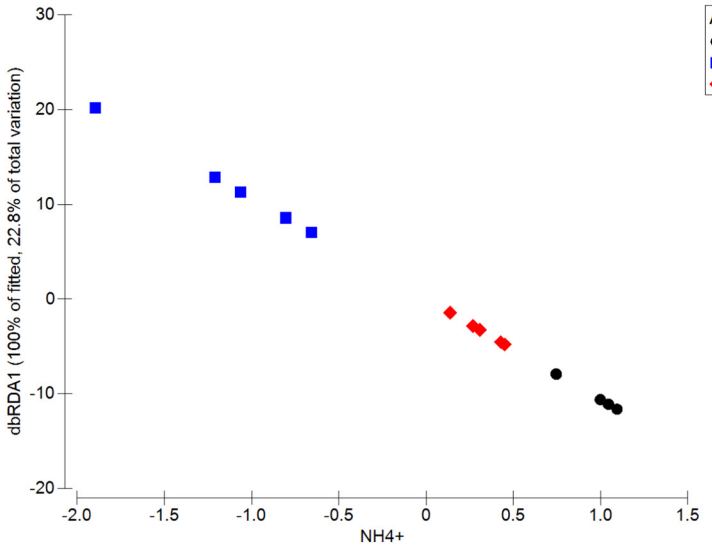

Fig. 4. Distance-based redundancy analysis (dbRDA) ordination plot of the DistLM sequential test results based on the soil abiotic variables fitted to the variation in the soil bacterial community. Soil NH_4^+ was the best predictor variable out of the model and explained 22.8% of the total variation observed in the soil bacterial community patterns across primary forest bulk-, *Dipteryx*-, and *Pentaclethra*-soil (Pseudo-F = 4.72, p = 0.0019, AICc = 110.87).

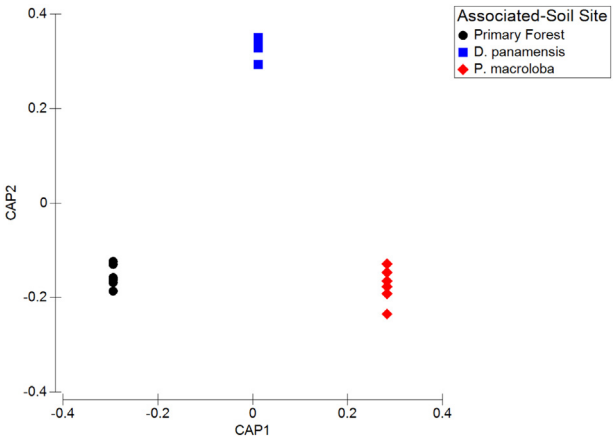

Fig. 5. Canonical Analysis of Principal Coordinates (CAP) showing the strength of the dissimilarity of the overall soil fungal community composition across the primary forest bulk soil, *Dipteryx*-soil, and *Pentaclethra*-soil in a primary old-growth lowland forest of Costa Rica. The CAP axis 1 squared canonical correlation was 0.9184 and the CAP axis 2 squared canonical correlation was 0.8178.

such as NH_4^+ and NO_3^-, but less so for the soil physico-chemical properties (i.e., soil *p*H and moisture content). As various plant growth processes mainly depend on inorganic and organic N uptake from the soil (Tegeder and Masclaux-Daubresse, 2018), the differences observed in this study could be attributed to that climax (shade-bearer) and pioneer (light-demander) tree species differ in terms of their principal source of inorganic N (Ghazoul and Sheil, 2010; Kricher, 2011). For example, climax tree species use NH_4^+ as the primary source of inorganic N, whereas pioneer tree species use NO_3^- (Ghazoul and Sheil, 2010; Kricher, 2011). As *Dipteryx* is an important climax tree species (Ghazoul and Sheil, 2010; Kricher, 2011) in the area of this study, it is possible that *Dipteryx* is using NH_4^+ as its primary source of inorganic N for vegetation growth processes for the plant, leading to less NH_4^+ in the *Dipteryx*-soils, and more allocation of NH_4^+ for plant growth mechanisms. On the other hand, given that *Pentaclethra* is a tropical pioneer species (and later successional species) (Ghazoul and Sheil, 2010; Kricher, 2011), it could be that this species is using NO_3^- as its principal source of inorganic N. Thus, this would result in less accumulation of NO_3^- in the immediately adjacent soils. Given the overall pattern here, the lower means of the NH_4^+ and NO_3^- and the differing demands for these nutrients by these plants, could suggest the inorganic N soil nutrient pools here represent the amount of NH_4^+ and NO_3^- left-over after utilization by the trees. However, it is important to note the

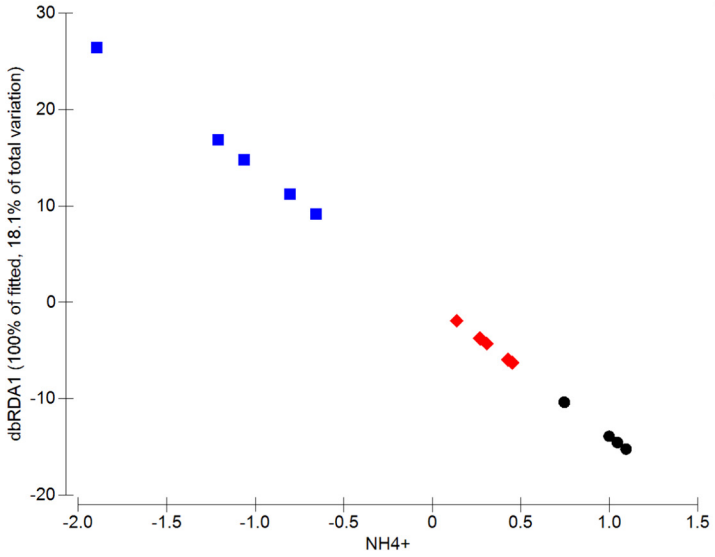

Fig. 6. Distance-based redundancy analysis (dbRDA) ordination plot of the DistLM sequential test results based on the soil abiotic variables fitted to the variation in the soil fungal community. Soil NH_4^+ was the best predictor variable out of the model and explained 18.1% of the total variation observed in the soil fungal community patterns across primary forest bulk-, *Dipteryx*-, and *Pentaclethra*-soils (Pseudo-F = 3.5389, p = 0.0007, AICc = 125.73).

current study measured the standing pools of soil NO_3^- and NH_4^+, and not the rate of production or utilization of these soil nutrients.

Moreover, *Pentaclethra*-soils had more C_{mic} than the non-N-fixing *Dipteryx*-soils and had similar amounts of C_{mic} to that of the primary forest-soils; suggesting that *Pentaclethra* stimulates the production of C_{mic} such that it is similar to the levels found in the primary forest-soil. This is most likely due to the N-fixing capabilities of *Pentaclethra*, and is consistent with previous evidence suggesting that trees with N-fixing symbioses can enhance soil biomass and soil organic matter levels (Eaton et al., 2012; Grant et al., 2007; Macedo et al., 2008) (but see, Hoogmoed et al., 2014a, 2014b, 2012). This could be corroborated by the finding that soil NH_4^+ explained almost 40% of the total variation observed in the soil C_{mic} patterns. Indeed, a greater nitrogen availability in the soil can increase a more efficient use of C in the soils, and as a result, more C remains in the soil in the form of microbial biomass and byproducts (Bradford and Crowther, 2013; Manzoni et al., 2012; Tucker et al., 2012; Vicca et al., 2012). However, if a tropical climax tree species is dependent on soil NH_4^+ as its principal source of inorganic N, that tree species would potentially be acting as an NH_4^+ sink with respect to its aboveground components, as has been observed elsewhere (Tegeder and Masclaux-Daubresse, 2018). Consequently, without soil NH_4^+ or a limited availability of NH_4^+ in soils beneath a tree such as *Dipteryx*, ammonium-oxidation could also become limiting, and potentially result in less soil C_{mic}. Thus, *Pentaclethra* could be acting as a source-dynamic for the facilitation of more soil C_{mic} in its soils, in contrast to *Dipteryx* which may be acting as a sink-dynamic that may ultimately result in less soil C_{mic} in its soils. Therefore, at the individual tree level, soil NH_4^+ may have a strong effect as the principal pathway in terms of promoting more C_{mic} (and thus, potentially more CUE) in primary forests in this area.

A recent meta-analysis exploring individual plant effects throughout the tropics, found that the largest effects were observed for base saturation, electrical conductivity, plant-available inorganic N, and others (Waring et al., 2015). Whereas plant effects on soil physico-chemical factors (e.g. soil bulk density, soil moisture, and soil *p*H) and total soil nutrient pools (e.g. soil TC, TN, C:N ratio) were less pronounced (Waring et al., 2015). Even though the meta-analysis was not examining N-fixing and non-N-fixing plants, inorganic N had one of the strongest effect sizes for influencing soil chemical properties. Thus, as inorganic N maybe be a strong driver of soil abiotic and biotic components, N-fixing or non-N-fixing tree species could have major consequences for soil C_{mic}. Our results are most consistent with the results of the meta-analysis in that the more limiting nutrients, such as inorganic N, had the strongest influence in terms of individual plant effects. However, in contrast, our results are inconsistent with previous studies examining N-fixing and non-N-fixing individual plant effects via monoculture plantations (Hoogmoed et al., 2014b; Kivlin and Hawkes, 2016a). For example, in two previous studies, no differences were observed for soil NH_4^+ (Hoogmoed et al., 2014b; Kivlin and Hawkes, 2016a), NO_3^- (Kivlin and Hawkes, 2016a), and C_{mic} (Kivlin and Hawkes, 2016a); and interestingly, the one monoculture plantation consisted of *Pentaclethra* (Kivlin and Hawkes, 2016a). Yet, in a study comparing *Pentaclethra*-soils to secondary forest bulk-soils, it was found that there was more soil C_{mic} associated with *Pentaclethra*-soils, than forest-soils (Eaton et al., 2012).

However, given the monoculture experimental design in some of these studies, it may be difficult to parse out and identify individual plant effects of N-fixing and non-N-fixing trees in a managed system. Thus, evaluating individual plant effects in an unmanaged ecosystem such as a primary forest still warrants more investigation. Overall, these results ultimately highlight that the native N-fixing tree species *Pentaclethra*, may be conducive for forest recuperation in comparison to the native non-N-fixing species, *Dipteryx*. In cases of large disparities in the amount of soil NH_4^+, large decreases or increases in NH_4^+ could be the soil abiotic driver that may ultimately determine the fate or direction of soil microbial biomass development. From this, it appears NH_4^+ may be quite important as the first step to building more microbial biomass in the soils, and that changes in the amount of NH_4^+ are acting as an environmental driver for soil C_{mic}.

4.2. Differences in the soil bacterial and fungal community composition

Our results demonstrated that the soil bacterial and fungal community compositions were significantly different between the tree-soils and from primary forest-soils. This suggests that individual plant effects influence the adjacent soil microbial community composition, which is different than the primary forest soil microbial composition. This shows that there could be a relatively strong influence a tree species can have on the surrounding soil microbiome in a highly mixed species tropical primary forest. Indeed, previous studies have shown that plants can shape the soil microbiome, particularly the rhizosphere communities, through the rhizodeposition of plant-root exudates, mucilage, and sloughed cells (Chaparro et al., 2012, 2014; Lakshmanan et al., 2014; Yan et al., 2017). Even though we did not directly sample and isolate the rhizosphere soils, our soil samples did consist of this soil habitat given our soil sampling design. Nonetheless, we predicted *Dipteryx*-, *Pentaclethra*-, and primary forest-soils to have their own distinct soil bacterial and fungal community composition due to individual plant effects that can affect different substrate qualities and quantities entering the soil; either through rhizodeposition (i.e. individual tree species plant roots) or through plant litter diversity and complexity on forest floor soils (i.e. forest soils).

The most notable pattern for the soil bacterial taxonomic groups were for those soil bacteria associated with N-cycling activities. For example, *Dipteryx*-soils had the greatest RPP of N-fixing bacteria (*Burkholderia* (spp.), *Bradyrhizobium* (spp.), and *Rhizomicrobium* (spp.)) (cumulative proportion 7.47%) compared to *Pentaclethra*-soils (cumulative proportion 4.92%). However, given that *Dipteryx* does not form N-fixing root nodule symbionts (Montagnini and Sancho, 1994), it is quite possible the greater cumulative proportion of N-fixing bacteria in *Dipteryx*-soils, compared to *Pentaclethra*-soils, are actually free-living N-fixing bacteria. The demand of soil NH_4^+ by *Dipteryx* could be driving the need for more free-living N-fixing bacteria, as a compensatory effect for the lack of N-fixing root nodule symbionts. The root nodular bacterial N-fixing symbionts are presumably more efficient in N-fixation processes as these bacterial symbionts have a steady stream of metabolic precursors from the plant, and therefore, less energy expenditure is required for biosynthesis. However, the free-living N-fixing bacteria need to expend more energy for biosynthesis, as these free-living bacteria do not have that steady stream of metabolic precursors from the plant, and thus, have less energy available for N-fixation. Therefore, the *Dipteryx* plant may require more free-living N-fixing bacteria in its soils to reach the functional contribution or efficiency to that of root nodular bacteria, in terms of producing soil NH_4^+. Moreover, the *Pentaclethra*-soils had the greatest RPP of the ammonium-oxidizing bacteria (AMoB) *Nitrospira* (7.3%) in contrast to *Dipteryx*-soils (3.2%). *Pentaclethra* would presumably need more AmoB in its soils to produce NO_3^- for utilization by the plant (as described earlier). In any case, it is difficult to ascribe whether there are more N-fixer populations due to the NH_4^+ demand of the *Dipteryx* plant species, or that more free-living N-fixing bacteria are required to compensate for the lack of root nodule microbial symbionts, or perhaps a combination of both. Therefore, it may be of interest in future studies to measure rates of N-fixation and ammonium-oxidation of these soils, as well as explore the quantity and expression of functional genes involved in N-cycling processes.

Similarly, we found N-fixing and non-N-fixing individual plant effects have influenced certain soil fungal taxonomic groups associated with N-cycling processes. For example, the *Pentaclethra*-soils harbored the most amount of the fungal yeast *Geotrichum* (51.7%) and had more than double the amount in comparison to *Dipteryx*-soils (15.95%). *Geotrichum* is not only able to synthesize carbohydrates in the soil but, can also nitrify NH_4^+ to NO_3^- (Al-Falih and Wainwright, 1995a,

1995b; Al-Falih, 2006; Botha, 2011), indicating important consequence for N-cycling activities within *Pentaclethra*-soils. In the case of *Pentaclethra*, this may most likely be to stimulate those microbes associated with nitrifying processes to produce soil NO_3^- that *Pentaclethra* may be using as its principal source of inorganic N. In addition, *Dipteryx*-soils had the most soil ECM (*Hygrocybe*) (1.71%), compared to *Pentaclethra*- and primary forest-soils (0.93% and 0.14%, respectively), and harbored the greatest proportion of *Archaeorhizomyces* (24%) that have been found around plant roots that may be dependent on soil C compounds from plant roots. The accumulation of NO_3^- associated with *Dipteryx* could be stimulating the growth of ECM, as has been observed elsewhere (Sinsabaugh, 2010; DeForest et al., 2004; de Vries et al., 2007; Knorr et al., 2005; Hobbie, 2008). Moreover, the soil ECM and *Archaeorhizomyces* associated with *Dipteryx* may be playing a crucial role in inorganic N nutrient acquisition and assimilation (Abuzinadah et al., 1986; Read and Perez-Moreno, 2003). It has been observed that in the presence of favorable sources of N such as NH_4^+, filamentous ECM fungi can exhibit repressed expression of proteolytic activity (Marzluf, 1996; Zhu et al., 1994). The reason for this is that the ECM have been shown to be important scavengers of organic forms of N, degrading these compounds so as to bring in these forms of N for use (Talbot et al., 2008). However, if excess inorganic N is already present, then higher levels of these scavenging activities are not needed and these levels will be suppressed.

Moreover, our findings also indicated that individual plant effects, whether with N-fixing capabilities or not, does not have an influence on soil bacterial and fungal genus diversity and richness. Previous studies have found similar results in that monoculture stands of different N-fixing and non-N-fixing tree species did not have an effect on fungal richness, and that bacterial richness depended more on sampling season (i.e. wetter vs drier seasons), rather than vegetation type (Kivlin and Hawkes, 2016a, 2016b). However, despite no differences in soil bacterial and fungal genus richness between the tree-soils, the soil bacterial and fungal genus richness were greatest in the primary forest-soils, and less in *Dipteryx*- and *Pentaclethra*-soils. This is most likely due to more abundant resources that would presumably be available for the soil microbial communities in primary forest bulk-soils as there would be less plant leaf litter in the soil adjacent to these tree species, in comparison to the primary forest floor. A greater multiplicity of resources and spatial heterogeneity (such as greater quantity, quality, and complexity of plant litter) on the forest floor would presumably create more niches or a partitioning of resources thereby reducing competition, and therefore, result in soil microbial communities with greater richness and greater biomass to consume these resources (Bradford et al., 2013, 2008, 2014; Guggenberger et al., 1995; Guggenberger and Zech, 1999; Schwendenmann and Veldkamp, 2006; Zhang and Zak, 1995).

4.3. Soil NH_4^+ as drivers of soil bacterial and fungal community composition

The bacterial and fungal communities in soils have strong connections to the surrounding environment and it is well known that various soil site factors such as *p*H, moisture, and texture, etc., can shape microbial community composition (Fierer et al., 2007; Fierer et al., 2009). However, a soil microbial population that is existing close to the edge of its upper or lower tolerance limit, grows rather slowly under these stressful conditions. Thus, those microbial populations become more vulnerable to ecological interactions such as competition, predation, and parasitism. Environmental conditions act as filter processes that should select for organisms according to their traits, niche preferences, biological interactions, and coevolution with hosts, and therefore, shape the microbial community compositions (Costello et al., 2012; McCalley et al., 2014; Nemergut et al., 2013). Yet, the environmental filters or drivers of certain functional implications are not well known. Recent findings suggest that fluctuations of key microbial taxa reflect the dynamics of important biogeochemical processes (McCalley et al., 2014). In less severe habitats, or a narrow range of environmental conditions, nutrient availability and substrate qualities and quantities, should exert a strong force on the biochemical and ecological interactions among populations, that would change the direction and trajectory of various ecosystem processes, such as decomposition (Atlas and Bartha, 1981). Ultimately, this may have consequences or various potential outcomes of ecosystem functioning (McCalley et al., 2014). The drivers of this acting on local-scales, such as here with individual plant effects, become important for understanding possible functional outcomes extrapolated to a habitat or forest ecosystem scale, particularly so for remediation efforts.

From our results, not only does it appears that NH_4^+ is important in structuring the soil bacterial and fungal communities, it is important in structuring a microbial community that associated with more microbial biomass in the soils. Thus, this may have potential consequences for improved CUE. Soil NH_4^+ as a driver of the bacterial and fungal community structure can be explained in part, by its role in the utilization by bacteria and fungi as an important preferred N-source. Forms of inorganic N have been known to stimulate the growth of soil ECM (Singh et al., 2008), but inhibit many of those fungi capable of complex recalcitrant C degradation (e.g. lignin, cellulose, hemicellulose) (DeForest et al., 2004; Waldrop and Firestone, 2006; Waldrop and Zak, 2006). Soil microbial decomposers with a greater CUE can convert substrates in the soil more efficiently to new biomass, leading to a reduced amount of respiration per unit of C take-up (Bradford and Crowther, 2013; Manzoni et al., 2012; Sinsabaugh et al., 2016). For example, in general, fungi have a greater CUE than bacteria, meaning that fungi can assimilate C more efficiently into biomass that involves less loss of C via maintenance respiration and requires more N for the enzymes needed for biosynthesis (Anderson, 2003; Anderson and Domsch, 2010; Brookes, 1995; Moscatelli et al., 2005; Strickland and Rousk, 2010; Waring et al., 2013). As such, this can have potential consequences for the amount of C stored in microbial biomass and other various forms, rather than C loss through respiratory maintenance processes (Manzoni et al., 2012; Sinsabaugh et al., 2016). In connection with the soil C_{mic} data, it could be that at the local-scale, the NH_4^+ source-dynamic of *Pentaclethra* is stimulating a bacterial and fungal decomposer community that is more efficient in the incorporation of C for biomass. Therefore, it appears as if less NH_4^+, is perhaps a more important limiting factor for microbial biomass production in the soils examined in this study, and that changes in the amount of NH_4^+ are acting as an environmental driver that can shape the soil bacterial and fungal community composition with varying decomposer efficiencies. Consequently, decreased levels of inorganic N could result in a decrease in the synthesis of these enzymes, and therefore, a decrease in the rate and efficiency of these complex decomposition activities. Ultimately, this would lead to a reduction in biomass, and potentially a reduction in CUE.

Our study contradicts many others in that *p*H was not a driver of soil microbial community composition (Banerjee et al., 2016; Castro et al., 2010; Freedman and Zak, 2015; Högberg et al., 2006; Kivlin and Hawkes, 2016b; Lee-Cruz et al., 2013; Sait et al., 2006; Tripathi et al., 2012). However, soil abiotic drivers such as *p*H (or others such as moisture, soil texture, etc.) that can shape community composition may not always have an effect on the functional process of the microbes. For example, Kivlin and Hawkes (2016b), found that soil moisture and *p*H accounted for ~4% of the variation in total soil fungal community structure. Yet *p*H was significantly different across their monoculture vegetation types, with *Pentaclethra* stands being the most acidic (*p*H = 4.2). As many microbes are capable of performing the same function, there may be small to large associated shifts in taxa with changing or different climatic and environmental variables, but there may be no change in the overall functional response of the microbial community. For example, even if soil *p*H is structuring the composition of two microbial communities between two habitats, this does not mean that there is an associated functional shift with a shift in community

composition. Thus, *p*H could shape communities but not necessarily have some sort of larger effect that changes the amount of soil microbial biomass C, as a result of structuring the microbial communities present.

5. Conclusions

Our study adds to the growing body of literature for individual plant effects in the tropics. The role of individual plant effects on soil conditions in structuring microbial community composition associated with N-fixing and non-N-fixing trees, and primary forest soils, has not previously been assessed in this region of Costa Rica. Tropical forests exchange CO_2 with the atmosphere than any other terrestrial ecosystem and are global drivers of soil carbon biomass (Jobbágy and Jackson, 2000; Nottingham et al., 2015), however, human-driven land-use changes and forest disturbances can have significance impacts on the carbon biomass that is stored in these soils, and thus, atmosphere CO_2 levels (Brienen et al., 2015; Laurance, 2007; Nottingham et al., 2015). A better understanding of the extent individual tree species influence soil microbial heterogeneity can help decision-making strategies for the selection of plant species for potential reforestation and remediation efforts. As such, if a particular tree species is capable of stimulating soil C_{mic} levels similar to that of a primary forest, that particular tree species could prove to be a good gateway tree species in which to facilitate remediation efforts. Moreover, if we can understand the soil microbial and environmental drivers associated with individual plant effects that are important for belowground biomass development, this could have important implications for the recuperation of degraded soils. Thus, the results from this study provide evidence that *Pentaclethra* could be a remediation tool for recuperating important sources of inorganic soil N as well as the development of soil C_{mic} in regenerating secondary forests in the area, but still warrants much more investigation.

This study showed that N-fixing and non-N-fixing individual plant effects have a strong influence on some of the abiotic properties involved with N-cycling processes, and that the soil C_{mic} and microbial communities are driven by these differences in soil NH_4^+. As such, in cases of reduced soil NH_4^+, decreases in NH_4^+ could be an important factor which ultimately determines the fate or direction of biomass development. This suggests that soil NH_4^+ may be structuring a more efficient soil microbial decomposer community capable of allocating C toward biomass as opposed to lose through respiratory processes. Even though this study only examined two different tree species in the area, the results have shown strong implications for *Pentaclethra* to stimulate microbial biomass C in primary forest soils than a non-N-fixing tree species of the same family. As such, this plant species could be an important ecosystem restoration tool used in facilitating early regeneration of secondary forests, providing essential forms of inorganic N that are important for plant and microbial growth and enhancing CUE. Therefore, this tree species may have a greater potential for facilitating biomass development, and possibly C sequestration, than native non-N-fixing species in the area. Future studies should focus on understanding how *Pentaclethra* influences soil biotic communities and C and N cycle dynamics in previously managed systems compared to old-growth forests, and a project concerning this is currently underway.

Acknowledgments

We would like to thank Vinzenz and Kurt Schmack, the staff members at the Laguna del Lagarto Lodge, and undergraduate student Olivia Karas for her assistance in the project and processing soil samples. This study was supported by grants from the Government of Canada through Environment and Climate Change Canada and NSERC to MH. This study was also supported by a grant from the National Science Foundation (DBI-1262907); Costa Rican Government Permit #063-2008-SINAC. TMP would like to acknowledge funding from the Canadian government through the Genomics Research and Development Initiative (GRDI) interdepartmental EcoBiomics project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2019.02.007.

References

Abrams, M.D., Hock, W.K., 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.
Abuzinadah, R.A., Finlay, B.J., Read, D.J., 1986. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. II. Utilizations of proteins by mycorrhizal plants of *Pinus contorta*. New Phytol. 103, 495–506.
Alef, K., Nannipieri, P., 1995. Methods in Applied Soil Microbiology and Biochemistry. Elsevier, Academic Press, London, UK.
Al-Falih, A.M., 2006. Nitrogen transformation in vitro by some soil yeasts. Saudi J. Biol. Sci. 13, 135e140.
Al-Falih, A.M., Wainwright, M., 1995a. Nitrification, S oxidation and P-solubilization by the soil yeast *Williopsis californica* and by *Saccharomyces cerevisiae*. Mycol. Res. 99, 200e204.
Al-Falih, A.M., Wainwright, M., 1995b. Nitrification in vitro by a range of filamentous fungi and yeasts. Lett. Appl. Microbiol. 21, 18e19.
Allen, O.N., Allen, E.K., 1981. The Leguminosae: A Source Book of Characteristics, Uses, and Nodulation. University of Wisconsin Press, Madison, Wisconsin (812 pp.).
Allison, S.D., Martiny, J.B.H., 2008. Colloquium paper: resistance, resilience, and redundancy in microbial communities. Proc. Natl. Acad. Sci. U. S. A. 105, 11512–11519. https://doi.org/10.1073/pnas.0801925105.
Anderson, T.H., 2003. Microbial eco-physiological indicators to assess soil quality. Agric. Ecosyst. Environ. 98, 285–293. https://doi.org/10.1016/S0167-8809(03)00088-4.
Anderson, T.H., Domsch, K.H., 2010. Soil microbial biomass: the eco-physiological approach. Soil Biol. Biochem. 42, 2039–2043. https://doi.org/10.1016/j.soilbio.2010.06.026.
Anderson, J.M., Ingram, J., 1993. Tropical Soil Biology and Fertility: A Handbook of Methods, 2nd ed. CAB International, Cambridge, MA.
Anderson, M.J., Willis, T.W., 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, M.J., Gorley, R.N., Clarke, R.K., 2008. PERMANOVA+ for Primer: Guide to Software and Statistical Methods. Plymouth, UK.
Asner, G.P., Rudel, T.K., Aide, T.M., DeFries, R., Emerson, R., 2009. A contemporary assessment of change in humid tropical forests. Conserv. Biol. 23, 1386–1395. https://doi.org/10.1111/j.1523-1739.2009.01333.x.
Atlas, R.M., Bartha, R., 1981. Microbial Ecology: Fundamentals and Applications. Addison-Wesley Publishing Company0201000512 (560 pp.).
Ayres, E., Steltzer, H., Simmons, B.L., Simpson, R.T., Steinweg, J.M., Wallenstein, M.D., Mellor, N., Parton, W.J., Moore, J.C., Wall, D.H., 2009. Home-field advantage accelerates leaf litter decomposition in forests. Soil Biol. Biochem. 41, 606–610. https://doi.org/10.1016/j.soilbio.2008.12.022.
Banerjee, S., Baah-Acheamfour, M., Carlyle, C.N., Bissett, A., Richardson, A.E., Siddique, T., Bork, E.W., Chang, S.X., 2016. Determinants of bacterial communities in Canadian agroforestry systems. Environ. Microbiol. 18, 1805–1816. https://doi.org/10.1111/1462-2920.12986.
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, M.R., Gregorich, E.G. (Eds.), Soil Sampling and Methods of Analysis, second edition. Taylor & Francis Group, LLC, Boca Raton, FL, pp. 39–46.
Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, I., de Sousa, F., Amend, A.S., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y.J.K., Sanli, K., Eriksson, K.M., Vik, U., Veldre, V., Nilsson, R.H., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol. Evol. 25https://doi.org/10.1111/2041-210X.12073. (n/a–n/a).
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.
Binkley, D., Menyailo, O., 2005. Gaining insights on the effects of tree species on soils. In: Tree Species Effects on Soils: Implications for Global Change. NATO Science Series IV: Earth and Environmental Sciences Springer, Dordrecht, Berlin/Heidelberg, pp. 1–16. https://doi.org/10.1007/1-4020-3447-4_1.
Botha, A., 2011. The importance and ecology of yeasts in soil. Biochemistry 43, 1–8.
Bradford, M.A., Crowther, T.W., 2013. Carbon use efficiency and storage in terrestrial ecosystems. New Phytol. 199, 7–9. https://doi.org/10.1111/nph.12334.
Bradford, M.A., Fierer, N., Reynolds, J.F., 2008. Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. Funct. Ecol. 22, 964–974. https://doi.org/10.1111/j.1365-2435.2008.01404.x.
Bradford, M.A., Keiser, A.D., Davies, C.A., Mersmann, C.A., Strickland, M.S., 2013. Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth. Biogeochemistry 113, 271–281. https://doi.org/10.1007/s10533-012-9822-0.
Bradford, M.A., Wood, S.A., Bardgett, R.D., Black, H.I.J., Bonkowski, M., Eggers, T.,

Grayston, S.J., Kandeler, E., Manning, P., Setälä, H., Jones, T.H., 2014. Discontinuity in the responses of ecosystem processes and multifunctionality to altered soil community composition. Proc. Natl. Acad. Sci. 111, 14478–14483. https://doi.org/10.1073/pnas.1413707111.

Brienen, R.J.W., Phillips, O.L., Feldpausch, T.R., Gloor, E., Baker, T.R., Lloyd, J., Lopez-Gonzalez, G., Monteagudo-Mendoza, A., Malhi, Y., Lewis, S.L., Vásquez Martinez, R., Alexiades, M., Álvarez Dávila, E., Alvarez-Loayza, P., Andrade, A., Aragão, L.E.O.C., Araujo-Murakami, A., Arets, E.J.M.M., Arroyo, L., Aymard C, G.A., Bánki, O.S., Baraloto, C., Barroso, J., Bonal, D., Boot, R.G.A., Camargo, J.L.C., Castilho, C.V., Chama, V., Chao, K.J., Chave, J., Comiskey, J.A., Cornejo Valverde, F., da Costa, L., de Oliveira, E.A., Di Fiore, A., Erwin, T.L., Fauset, S., Forsthofer, M., Galbraith, D.R., Grahame, E.S., Groot, N., Hérault, B., Higuchi, N., Honorio Coronado, E.N., Keeling, H., Killeen, T.J., Laurance, W.F., Laurance, S., Licona, J., Magnussen, W.E., Marimon, B.S., Marimon-Junior, B.H., Mendoza, C., Neill, D.A., Nogueira, E.M., Núñez, P., Pallqui Camacho, N.C., Parada, A., Pardo-Molina, G., Peacock, J., Peña-Claros, M., Pickavance, G.C., Pitman, N.C.A., Poorter, L., Prieto, A., Quesada, C.A., Ramírez, F., Ramírez-Angulo, H., Restrepo, Z., Roopsind, A., Rudas, A., Salomão, R.P., Schwarz, M., Silva, N., Silva-Espejo, J.E., Silveira, M., Stropp, J., Talbot, J., ter Steege, H., Teran-Aguilar, J., Terborgh, J., Thomas-Caesar, R., Toledo, M., Torello-Raventos, M., Umetsu, R.K., van der Heijden, G.M.F., van der Hout, P., Guimarães Vieira, I.C., Vieira, S.A., Vilanova, E., Vos, V.A., Zagt, R.J., 2015. Long-term decline of the Amazon carbon sink. Nature 519, 344–348. https://doi.org/10.1038/nature14283.

Brookes, P.C., 1995. The use of microbial parameters in monitoring soil pollution by heavy metals. Biol. Fertil. Soils 19, 269–279. https://doi.org/10.1007/BF00336094.

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

Burnham, K.P., Anderson, D.R., 1998. Model selection and multi-model inference: a practical information-theoretical approach. In: Model Selection and Inference, 2nd ed. pp. 75–117. https://doi.org/10.1007/978-1-4757-2917-7_3.

Calvo-Alvarado, J.C., Arias, D., Richter, D.D., 2007. Early growth performance of native and introduced fast growing tree species in wet to sub-humid climates of the Southern region of Costa Rica. For. Ecol. Manag. 242, 227–235. https://doi.org/10.1016/j.foreco.2007.01.034.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., 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.

Carter, R., Lowe, L., 1986. Lateral variability for forest floor properties under second-growth Douglas-fir stands and the usefulness of composite sampling techniques. Can. J. For. Res. 16, 1128–1132.

Castro, H.F., Classen, A.T., Austin, E.E., Norby, R.J., Schadt, C.W., 2010. Soil microbial community responses to multiple experimental climate change drivers. Appl. Environ. Microbiol. 76, 999–1007. https://doi.org/10.1128/AEM.02874-09.

Chaparro, J.M., Sheflin, A.M., Manter, D.K., Vivanco, J.M., 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, J.M., Badri, D.V., Vivanco, J.M., 2014. Rhizosphere microbiome assemblage is affected by plant development. ISME J. 8, 790–803. https://doi.org/10.1038/ismej.2013.196.

Chassot, O., Monge, G., 2012. Connectivity conservation of the great green macaw's landscape in Costa Rica and Nicaragua (1994-2012). Parks 18, 1–10.

Claesson, M.J., O'Sullivan, O., Wang, Q., Nikkilä, J., Marchesi, J.R., Smidt, H., de Vos, W.M., Ross, R.P., O'Toole, P.W., 2009. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. PLoS One 4https://doi.org/10.1371/journal.pone.0006669. (e6669–15).

Clarke, K.R., 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, K.R., Gorley, R.N., 2006. Plymouth: Primer-E Ltd. PRIMER v6: User Manual/Tutorial (Plymouth Routines in Multivariate Ecological Research).

Costello, E.K., Stagaman, K., Dethlefsen, L., Bohannan, B.J.M., Relman, D.A., 2012. The application of ecological theory toward an understanding of the human microbiome. Science 336, 1255–1262. https://doi.org/10.1126/science.1224203.

Cove, M.V., Spínola, R.M., Jackson, V.L., Saénz, J.C., 2014. The role of fragmentation and landscape changes in the ecological release of common nest predators in the Neotropics. PeerJ 2, e464. https://doi.org/10.7717/peerj.464.

Cusack, D., Montagnini, F., 2004. The role of native species plantations in recovery of understory woody diversity in degraded pasturelands of Costa Rica. For. Ecol. Manag. 188, 1–15. https://doi.org/10.1016/S0378-1127(03)00302-5.

de Vries, F.T, Bloem, J., Van Eekeren, N., Brussaard, L., Hoffland, E., 2007. Fungal biomass in pastures increases with age and reduced N input (Soil Biol. Biochem.). 39, 1620–1630.

DeForest, J.L., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2004. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. Soil Sci. Soc. Am. J. 68, 132–138. https://doi.org/10.2136/sssaj2004.1320.

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, W.D., Anderson, C., Saunders, E.F., Hauge, J.B., Barry, D., 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.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Enquist, B.J., Leffler, A.J., 2001. Long-term tree ring chronologies from sympatric tropical dry-forest trees: Individualistic responses to climatic variation. J. Trop. Eco. 17 (1), 41–60.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364. https://doi.org/10.1890/05-1839.

Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. Ecol. Lett. 12, 1238–1249. https://doi.org/10.1111/j.1461-0248.2009.01360.x.

Foster, D., Swanson, F., Aber, J., Burke, I., Brokaw, N., Tilman, D., Knapp, A., 2003. The importance of land-use legacies to ecology and conservation. Bioscience 53, 77–88.

Freedman, Z., Zak, D.R., 2015. Soil bacterial communities are shaped by temporal and environmental filtering: evidence from a long-term chronosequence. Environ. Microbiol. 17, 3208–3218. https://doi.org/10.1111/1462-2920.12762.

French, D.W., Juzwik, J., 1999. Oak Wilt in Minnesota (MI-3174). University of Minnesota, Minnesota Extension Service, St. Paul, MN (6 pp.).

Gardes, M., Bruns, T.D., 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, P.L.G., de Souza, L.A.G., 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, M.G., Powers, J.S., 2013. Do legumes and non-legume tree species affect soil properties in unmanaged forest and plantations in Costa Rican dry forests? Soil Biol. Biochem. 57, 264–272. https://doi.org/10.1016/j.soilbio.2012.09.013.

Ghazoul, J., Sheil, D., 2010. Tropical Rain Forest Ecology, Diversity, and Conservation. Oxford University Press0199285888 (496 pp.).

Gibbs, H.K., Ruesch, A.S., Achard, F., Clayton, M.K., Holmgren, P., Ramankutty, N., Foley, J.A., 2010. Tropical forests were the primary sources of new agricultural land in the 1980s and 1990s. Proc. Natl. Acad. Sci. 107, 16732–16737. https://doi.org/10.1073/pnas.0910275107.

Gougoulias, C., Clark, J.M., Shaw, L.J., 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.

Grant, C.D., Ward, S.C., Morley, S.C., 2007. Return of ecosystem function to restored bauxite mines in Western Australia. Restor. Ecol. 15, S94–S103.

Guariguata, M.R., 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.

Guggenberger, G., Zech, W., 1999. Soil organic matter composition under primary forest, pasture, and secondary forest succession, Región Huetar Norte, Costa Rica. For. Ecol. Manag. 124, 93–104. https://doi.org/10.1016/S0378-1127(99)00055-9.

Guggenberger, G., Zech, W., Thomas, R.J., 1995. Lignin and carbohydrate alteration in particle-size separates of an oxisol under tropical pastures following native savanna. Soil Biol. Biochem. 27, 1629–1638. https://doi.org/10.1016/0038-0717(95)00080-X.

Halliday, J., 1984. Register of nodulation reports for leguminous trees and other arboreal genera within nitrogen fixing members. In: Nitr Fix. Tree Res. Reports. vol. 2. pp. 38–45.

Harrison, K.A., Bardgett, R.D., 2010. Influence of plant species and soil conditions on plant-soil feedback in mixed grassland communities. J. Ecol. 98, 384–395. https://doi.org/10.1111/j.1365-2745.2009.01614.x.

Hart, S.C., Binkley, D., Perry, D.A., 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, G.S., Hammel, B., 1994. Vegetation types and floristic patterns. In: La Selva: Ecology and Natural History of a Neotropical Rain Forest. The University of Chicago Press, Chicago, IL.

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

Hobbie, S.E., 2008. Nitrogen effects on decomposition: a five-year experiment in eight temperate sites. Ecology 89, 2633–2644.

Högberg, M.N., Högberg, P., Myrold, D.D., 2006. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150, 590–601. https://doi.org/10.1007/s00442-006-0562-5.

Holl, K.D., 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.

Hoogmoed, M., Cunningham, S.C., Thomson, J.R., Baker, P.J., Beringer, J., Cavagnaro, T.R., 2012. Does afforestation of pastures increase sequestration of soil carbon in Mediterranean climates? Agric. Ecosyst. Environ. 159, 176–183. https://doi.org/10.1016/j.agee.2012.07.011.

Hoogmoed, M., Cunningham, S.C., Baker, P., Beringer, J., Cavagnaro, T.R., 2014a. N-fixing trees in restoration plantings: effects on nitrogen supply and soil microbial communities. Soil Biol. Biochem. 77, 203–212. https://doi.org/10.1016/j.soilbio.2014.06.008.

Hoogmoed, M., Cunningham, S.C., Baker, P.J., Beringer, J., Cavagnaro, T.R., 2014b. Is there more soil carbon under nitrogen-fixing trees than under non-nitrogen-fixing trees in mixed-species restoration plantings? Agric. Ecosyst. Environ. 188, 80–84. https://doi.org/10.1016/j.agee.2014.02.013.

Höper, H., 2006. Substrate-induced respiration. In: Bloem, J., Hopkins, D.W., Benedetti, A. (Eds.), Microbiological Methods for Assessing Soil Quality. CAB International, Cambridge, MA, pp. 84–92.

Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol. Appl. 10, 423–426. https://doi.org/10.

2307/2641104.
Jacoby, G.C., D'Arrigo, R., 1990. Teak (*Tectona grandis* L.f.), a tropical species of large-scale dendroclimatic potential. Dendrochronologia 8, 83–98.
Kardol, P., Wardle, D.A., 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.
Ke, P.-J., Miki, T., 2015. Incorporating the soil environment and microbial community into plant competition theory. Front. Microbiol. 6https://doi.org/10.3389/fmicb.2015.01066. (438–16).
Kimmins, J.P., Comeau, P.G., Kurz, W., 1990. Modeling the interactions between moisture and nutrients in the control of forest growth. For. Ecol. Manag. 30, 361–379.
Kivlin, S.N., Hawkes, C.V., 2016a. Temporal and spatial variation of soil bacteria richness, composition, and function in a neotropical rainforest. PLoS One 11https://doi.org/10.1371/journal.pone.0159131. (e0159131–17).
Kivlin, S.N., Hawkes, C.V., 2016b. Tree species, spatial heterogeneity, and seasonality drive soil fungal abundance, richness, and composition in Neotropical rainforests. Environ. Microbiol. 18, 4662–4673. https://doi.org/10.1111/1462-2920.13342.
Knorr, M., Frey, S.D., Curtis, P.S., 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86, 3252–3257.
Kricher, J., 2011. Tropical Ecology. Princeton University Press9780691115139.
Lakshmanan, V., Selvaraj, G., Bais, H.P., 2014. Functional soil microbiome: belowground solutions to an aboveground problem. Plant Physiol. 166, 689–700. https://doi.org/10.1104/pp.114.245811.
Laurance, W.F., 2007. Have we overstated the tropical biodiversity crisis? Trends Ecol. Evol. 22, 65–70. https://doi.org/10.1016/j.tree.2006.09.014.
Lee-Cruz, L., Edwards, D.P., Tripathi, B.M., Adams, J.M., 2013. Impact of logging and forest conversion to oil palm plantations on soil bacterial communities in Borneo. Appl. Environ. Microbiol. 79, 7290–7297. https://doi.org/10.1128/AEM.02541-13.
Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 69, 1–24. https://doi.org/10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2.
Liu, K.-L., Porras-Alfaro, A., Kuske, C.R., Eichorst, S.A., Xie, G., 2012. Accurate, rapid taxonomic classification of fungal large-subunit rRNA genes. Appl. Environ. Microbiol. 78, 1523–1533. https://doi.org/10.1128/AEM.06826-11.
Macedo, M.O., Resende, A.S., Garcia, P.C., Boddey, R.M., Jantalia, C.P., Urquiaga, S., Campello, E.F.C., Franco, A.A., 2008. Changes in soil C and N stocks and nutrient dynamics 13 years after recovery of degraded land using leguminous nitrogen-fixing trees. For. Ecol. Manag. 255, 1516–1524. https://doi.org/10.1016/j.foreco.2007.11.007.
Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol. 196, 79–91. https://doi.org/10.1111/j.1469-8137.2012.04225.x.
Martin, M., 2011. Cutadapt Removes Adapter Sequences From High-Throughput Sequencing Reads. EMBnet.journal, [S.l.] 17 (1), 10–12. Available at: http://journal.embnet.org/index.php/embnetjournal/article/view/200.
Marzluf, G.A., 1996. Regulation of nitrogen metabolism in mycelial fungi. In: Brambl, B., Marzluf, G.A. (Eds.), The Mycota III. Biochemistry and Molecular Biology. Springer, Berlin.
Matheny, N., Clark, J.R., 1998. Trees and Development—A Technical Guide to Preservation of Trees During Land Development. International Society of Arboriculture, Champaign, IL, USA.
McCalley, C.K., Woodcroft, Ben J., Hodgkins, S.B., Wehr, R.A., Kim, E.-H., Mondav, R., Crill, P.M., Chanton, J.P., Rich, V.I., Tyson, G.W., Saleska, S.R., 2014. Methane dynamics regulated by microbial community response to permafrost thaw. Nat. Publ. Group 514, 478–481. https://doi.org/10.1038/nature13798.
McGee, K.M., Eaton, W.D., Shokralla, S., Hajibabaei, M., 2019. 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. 77 (1), 148–167.
McGuire, K.L., Zak, D.R., Edwards, I.P., Blackwood, C.B., Upchurch, R., 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.
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.
Monge, G.M., Chassot, O., Vargas, R.L., Kiel, H.C., 2002. Justificación biológica para el establecimiento del Parque Nacional Maquenque, Costa Rica: Corredor Biológico San Juan-La Selva. Centro Científico Tropical, pp. 1–51.
Montagnini, F., Sancho, F., 1994. Net nitrogen mineralization in soils under six indigenous tree species, an abandoned pasture and a secondary forest in the Atlantic lowlands of Costa Rica. Plant Soil 162, 117–124. https://doi.org/10.1007/BF01416097.
Moscatelli, M.C., Lagomarsino, A., Marinari, S., De Angelis, P., Grego, S., 2005. Soil microbial indices as bioindicators of environmental changes in a poplar plantation. Ecol. Indic. 5, 171–179. https://doi.org/10.1016/j.ecolind.2005.03.002.
Mukhopadhyay, S., Joy, V.C., 2010. Influence of leaf litter types on microbial functions and nutrient status of soil: ecological suitability of forest trees for afforestation in tropical laterite wastelands. Soil Biol. Biochem. 42, 2306–2315. https://doi.org/10.1016/j.soilbio.2010.09.007.
Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., 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, J.D., Carpenter, F.L., 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.
Nottingham, A.T., Whitaker, J., Turner, B.L., Salinas, N., Zimmermann, M., Malhi, Y., Meir, P., 2015. Climate Warming and Soil Carbon in Tropical Forests: Insights From an Elevation Gradient in the Peruvian Andes. vol. 65. pp. 906–921. https://doi.org/10.1093/biosci/biv109.
Pennock, D., 2004. Designing field studies in soil science. Can. J. Soil Sci. 84, 1–10.
Pennock, D., Yates, T., Braidek, J., 2006. Soil sampling designs. In: Carter, M.R., Gregorich, E.G. (Eds.), Soil Sampling and Methods of Analysis, second edition. Taylor & Francis Group, LLC, Boca Raton, FL, pp. 25–38.
Perry, T.O., 1982. The ecology of tree roots and the practical significance thereof. J. Arboric. 8, 197–211.
Pons, T.L., Perreijn, K., Van Kessel, C., Werger, M.J.A., 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, J.S., Haggar, J.P., Fisher, R.F, 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.
Prescott, C.E., Grayston, S.J., 2013. Tree species influence on microbial communities in litter and soil: current knowledge and research needs. For. Ecol. Manag. 309, 19–27. https://doi.org/10.1016/j.foreco.2013.02.034.
Raich, J.W., Clark, D.A., Schwendenmann, L., Wood, T.E., 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.
Read, D.J., Perez-Moreno, J., 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? New Phytol. 157, 475–492.
Reeder, J., Knight, R., 2009. The "rare biosphere": a reality check. Nat. Methods 6, 636–637. https://doi.org/10.1038/nmeth0909-636.
Sait, M., Davis, K.E.R., Janssen, P.H., 2006. Effect of pH on isolation and distribution of members of subdivision 1 of the phylum acidobacteria occurring in soil. Appl. Environ. Microbiol. 72, 1852–1857. https://doi.org/10.1128/AEM.72.3.1852-1857.2006.
Schwendenmann, L., Veldkamp, E., 2006. Long-term CO_2 production from deeply weathered soils of a tropical rain forest: evidence for a potential positive feedback to climate warming. Glob. Chang. Biol. 12, 1878–1893. https://doi.org/10.1111/j.1365-2486.2006.01235.x.
Shebitz, D.J., Eaton, W., 2013. Forest structure, nutrients, and Pentaclethra macroloba growth after deforestation of Costa Rican lowland forests. ISRN Ecol. 2013, 1–10. https://doi.org/10.1155/2013/414357.
Siddique, I., Engel, V.L., Parrotta, J.A., Lamb, D., Nardoto, G.B., Ometto, J.P.H.B., Martinelli, L.A., Schmidt, S., 2008. Dominance of legume trees alters nutrient relations in mixed species forest restoration plantings within seven years. Biogeochemistry 88, 89–101. https://doi.org/10.1007/s10533-008-9196-5.
Singh, B.K., Nunan, N., Ridgway, K.P., McNicol, J., Young, J.P.W., Daniell, T.J., Prosser, J.I., Millard, P., 2008. Relationship between assemblages of mycorrhizal fungi and bacteria on grass roots. Environ. Microbiol. 10, 534–541. https://doi.org/10.1111/j.1462-2920.2007.01474.x.
Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42, 391–404.
Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R., Moorhead, D.L., Shah, J.J.F., 2016. Stoichiometry of microbial carbon use efficiency in soils. Ecol. Monogr. 86, 172–189. https://doi.org/10.1890/15-2110.1.
Strickland, M.S., Rousk, J., 2010. Considering fungal: bacterial dominance in soils – methods, controls, and ecosystem implications. Soil Biol. Biochem. 42, 1385–1395. https://doi.org/10.1016/j.soilbio.2010.05.007.
Sundquist, A., Bigdeli, S., Jalili, R., Druzin, M.L., Waller, S., Pullen, K.M., El-Sayed, Y.Y., Taslimi, M.M., 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.
Talbot, J.M., Allison, S.D., Treseder, K.K., 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Funct. Ecol. 22, 955–963. https://doi.org/10.1111/j.1365-2435.2008.01402.x.
Tedersoo, L., Nilsson, R.H., Abarenkov, K., Jairus, T., Sadam, A., Saar, I., Bahram, M., Bechem, E., Chuyong, G., Kõljalg, U., 2010. 454 Pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. New Phytol. 188, 291–301. https://doi.org/10.1111/j.1469-8137.2010.03373.x.
Tegeder, M., Masclaux-Daubresse, C., 2018. Source and sink mechanisms of nitrogen transport and use. New Phytol. 217, 35–53. https://doi.org/10.1111/nph.14876.
Tripathi, B.M., Kim, M., Singh, D., Lee-Cruz, L., Lai-Hoe, A., Ainuddin, A.N., Go, R., Rahim, R.A., Husni, M.H.A., Chun, J., Adams, J.M., 2012. Tropical soil bacterial communities in Malaysia: pH dominates in the equatorial tropics too. Microb. Ecol. 64, 474–484. https://doi.org/10.1007/s00248-012-0028-8.
Tucker, C.L., Bell, J., Pendall, E., Ogle, K., 2012. Does declining carbon-use efficiency explain thermal acclimation of soil respiration with warming? Glob. Chang. Biol. 19, 252–263. https://doi.org/10.1111/gcb.12036.
Ushio, M., Wagai, R., Balser, T.C., 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, C.J., Gosling, P., Tiwari, B., Bending, G.D., 2010. Spatial scaling of arbuscular mycorrhizal fungal diversity is affected by farming practice. Environ. Microbiol. 13, 241–249. https://doi.org/10.1111/j.1462-2920.2010.02326.x.
Vicca, S., Luyssaert, S., Peñuelas, J., Campioli, M., Chapin III, F.S., Ciais, P., Heinemeyer,

A., Högberg, P., Kutsch, W.L., Law, B.E., Malhi, Y., Papale, D., Piao, S.L., Reichstein, M., Schulze, E.D., Janssens, I.A., 2012. Fertile forests produce biomass more efficiently. Ecol. Lett. 15, 520–526. https://doi.org/10.1111/j.1461-0248.2012.01775.x.

Waldrop, M.P., Firestone, M.K., 2006. Response of microbial community composition and function to soil climate change. Microb. Ecol. 52, 716–724. https://doi.org/10.1007/s00248-006-9103-3.

Waldrop, M.P., Zak, D.R., 2006. Response of oxidative enzyme activities to nitrogen deposition affects soil concentrations of dissolved organic carbon. Ecosystems 9, 921–933. https://doi.org/10.1007/s10021-004-0149-0.

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. https://doi.org/10.1128/AEM.00062-07.

Wardle, D.A., 2002. Communities and Ecosystems. Princeton University Press.

Wardle, D.A., 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, D.A., Jonsson, M., 2014. Long-term resilience of above- and belowground ecosystem components among contrasting ecosystems. Ecology 95, 1836–1849. https://doi.org/10.1890/13-1666.1.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633. https://doi.org/10.1126/science.1094875.

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. https://doi.org/10.1111/ele. 12125.

Waring, B.G., Álvarez-Cansino, L., Barry, K.E., Becklund, K.K., Dale, S., Gei, M.G., Keller, A.B., Lopez, O.R., Markesteijn, L., Mangan, S., Riggs, C.E., Rodríguez-Ronderos, M.-E., Segnitz, R.M., Schnitzer, S.A., Powers, J.S., 2015. Pervasive and strong effects on soil chemistry: a meta-analysis of individual plant 'Zinke' effects. Proc. R. Soc. B 282, 1–8. https://doi.org/10.1098/rspb2015.1001.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, N., Gelfand, D., Sninsky, J., White, T. (Eds.), PCR Protocols. NY, New York, pp. 315–322.

Whiting, D., 2013. Colorado Master Gardener GardenNotes. www.cmg.colostate.edu.

Yan, Y., Kuramae, E.E., de Hollander, M., Klinkhamer, P.G.L., van Veen, J.A., 2017. Functional traits dominate the diversity-related selection of bacterial communities in the rhizosphere. ISME J. 11, 56–66. https://doi.org/10.1038/ismej.2016.108.

Zhang, Q., Zak, J.C., 1995. Effects of gap size on litter decomposition and microbial activity in a subtropical forest. Ecology 76, 2196–2204. https://doi.org/10.2307/1941693.

Zhu, H., Dancik, B.P., Higginbotham, K.O., 1994. Regulation of extracellular proteinase production in an ectomycorrhizal fungus *Hebeloma crustuliniforme*. Mycologia 86, 227–234.

Zinke, P.J., 1962. The pattern of influence of individual forest trees on soil properties. Ecology 43, 130–133.