Microbial Ecology

The Influence of Tropical Plant Diversity and Composition on Soil Microbial Communities

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Abstract

There is growing interest in understanding the linkages between above- and belowground communities, and very little is known about these linkages in tropical systems. Using an experimental site at La Selva Biological Station, Costa Rica, we examined whether plant diversity, plant community composition, and season influenced microbial communities. We also determined whether soil characteristics were related to differences in microbial communities. Phospholipid fatty acid (PLFA) composition revealed that microbial community composition differed across a plant diversity gradient (plots contained 1, 3, 5, or over 25 species). Plant species identity also was a factor influencing microbial community composition; PLFA composition significantly varied among monocultures, and among three-species combinations that differed in plant species composition. Differences among treatments within each of these comparisons were apparent in all four sampling dates of the study. There was no consistent shift in microbial community composition between wet and dry seasons, although we did see significant changes over time. Of all measured soil characteristics, soil C/N was most often associated with changes in microbial community composition across treatment groups. Our findings provide evidence for human alteration of soil microbial communities via the alteration of plant community composition and diversity and that such changes are mediated in part by changes in soil carbon quality.

Introduction

Land-use change represents the most substantial human alteration of ecosystems on earth, dramatically altering plant communities and typically lowering biological diversity [55]. Although the direct effects of changes in plant community composition and/or diversity on ecosystem processes have been evaluated in a number of studies [24, 31, 32, 49–51], little is known about how microbes, key mediators of ecosystem processes, respond to changes in plant diversity or composition. This is because traditional descriptions of microbial communities have depended on techniques that required growth of microorganisms in the laboratory, which are now known to capture a scant proportion of the microbial species that persist in nature [27, 52, 53].

However, biomarker-based techniques, such as phospholipid and DNA analyses, enable detection of nonculturable species and allow a more complete and detailed picture of microbial communities [47, 54]. The use of phospholipids has become particularly popular in the past decade due to their ability to detect patterns of microbial community composition in the environment [46, 62] and the ease with which the data are collected relative to DNA methods. Although the detection of individual species is not possible with this method, phospholipid analyses allow one to investigate changes in broad microbial groups, such as fungi, bacteria, and actinomycetes [54]. Microbial ecologists have begun to apply these techniques to understanding the links between above- and belowground diversity [19, 30, 58].

Using biomarkers such as phospholipids and DNA, a number of investigators have found that agriculture significantly affects microbial community composition in both the temperate zone [7, 10, 28] and the tropics [5, 33, 56]. Similarly, recent work suggests that microbial community composition can be affected by plant species or even different cultivars of the same species in agricultural systems [3, 4, 30]. Few studies, however, have examined how microbial communities change in response to plant diversity using culture-independent techniques [58,

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61]. Furthermore, most of the studies above have been conducted in greenhouses, agricultural fields, or temperate grasslands; no studies to date have examined these issues in the tropics, where current anthropogenic alterations of plant community composition and diversity are most intense.

Changes in plant diversity and community composition could influence the composition of microbial communities through various direct and indirect mechanisms. Differences in plant communities can directly affect soil microbial communities through variations in the type, complexity, and amount of organic matter input to soils. Plant characteristics (e.g., rooting depth and density, canopy cover) also vary and can indirectly affect microbes via changes in the soil environment (e.g., soil moisture, temperature, and pH [1, 23]). Changes in any of these factors can influence physical and metabolic niche diversity in the soil, and therefore may affect microbial diversity or composition.

To examine these issues, we took advantage of an existing long-term experiment that directly manipulates plant composition and diversity while holding most other environmental variables constant [21]. Although the experiment was originally established to study the productivity and sustainability of tree plantations, we used the site design to understand how plant community composition and diversity affect microbial community composition. Plant species and communities in this experimental system have been shown to differ in productivity, litter chemistry, and rooting depth and density [20–22, 39], and we expected these differences to influence soil microbial communities. In this study, we test three specific hypotheses:

1. Microbial community composition differs across the plant diversity gradient and among plant communities that differ in composition. Studies have shown that plant species affect the composition of soil microbial communities; the differences among plant communities in litter and root production noted above will likely influence the abundance and composition of soil microorganisms.
2. Soil carbon content and quality are the soil characteristics most highly correlated to changes in microbial community composition. Most soil microorganisms are heterotrophs and depend on organic matter for energy, which is likely to make them sensitive to changes in the quantity and quality of soil organic matter. A previous study, which included a subset of the plots examined here, showed that plant communities differed in plant root production and quality and that these differences affected soil carbon quantity and quality [39]; we expected these plant-mediated changes in soil carbon to drive differences in soil microbial communities.
3. Microbial community composition differs between wet and dry seasons. Microbes have different tolerances to water stress and microbial composition can be indirectly affected via changes in nutrient supply due to moisture-related changes in microbial activity. Previous studies have shown changes in microbial communities between seasons and across time within a given season [6, 19, 45], but little is known regarding the sensitivity of soil microbes to seasonality in wet tropical forests.

Methods

Study System. We conducted this study within an experimental system located at La Selva Biological Station in the Atlantic tropical lowlands of Costa Rica; a complete description of the study system is presented in Haggar and Ewel [21]. The study system is located on an alluvial terrace that sustained cacao plantations for over 30 years prior to the experiment. In 1991, after all cacao vegetation was cut and the slash burned, plant communities were established that varied in species composition, diversity, and disturbance regime in a split-plot randomized block design [39]. We sampled a subset of plant communities from the experimental system that spanned four levels of plant diversity (Table 1). Three fast-growing hardwood species that differ in phenology and morphological characteristics (*Hyeronima alchorneoides*, *Cedrela odorata*, and *Cordia alliodora*) were grown in monoculture (three replicates each; 40 × 30 m). Each of these hardwoods was also planted with two other species, an herb (*Heliconia imbricata*) and a palm (*Euterpe oleracea*), for a total of three different three-species combinations (three replicates each; 40 × 30 m). All three species grown in monoculture were also combined with two other palm species to create a five-species combination (two replicates; 40 × 40 m). Finally, two plots were allowed to regenerate naturally, resulting in high plant species diversity (>25 species; two replicates of irregular shape; sampled from 30 × 30 m within each plot). In all but the secondary succession plots, trees were planted 2 m apart from conspecifics and were subsequently thinned to maximize resource use and minimize competition (J. Ewel, pers. comm. [21]).

The sandy loam soils of the site are classified as mixed, isohyperthermic, andic, fluventic Dysotropept. Mean annual rainfall is 4 m, and mean annual temperature is 24°C. There is a short dry season between February and April, although mean monthly rainfall never falls below 0.1 m [21].

Sampling. We sampled soils from each plot in two different wet seasons (November 2000 and November 2001) and dry seasons (April 2000 and April 2002). In each field replicate, we took 10 random samples of soil to 10-cm depth using a 2.5-cm diameter soil auger. No attempt was

Table 1. Design of the experimental system used in this study

Plot type	*Monoculture*			*Three-species combination*			*Five-species combination*	*Secondary succession*
Composition	Sp. 1	Sp. 2	Sp. 3	Sp. 1 + herb + palm 1	Sp. 2 + herb + palm 1	Sp. 3 + herb + palm 1	Spp. 1, 2, 3 + palm 1 + palm 2	>25 naturally occurring species
Replicates	3	3	3	3	3	3	2	2

Sp. 1 = *H. alchorneoides*, Sp. 2 = *C. odorata*, Sp. 3 = *C. alliodora*, herb = *H. imbricata*, palm 1 = *E. oleracea*, and palm 2 = *Euterpe macrospadix*.

made to separate rhizosphere and bulk soil. We pooled and homogenized the 10 cores (i.e., we had one mixed soil sample for each field replicate) and hand-sieved soil to remove large pieces of organic material (i.e., 2 mm or greater in size). Soils were transported on ice to Stanford University for analysis. Soil nitrogen analyses were immediately conducted (within 5 days of soil sampling) and subsamples of soil for microbial community analysis were frozen for analysis at a later date. Subsamples of the remaining soil were either air-dried for soil pH and phosphorus analyses, dried at 60°C for carbon analyses, or dried at 105°C for soil moisture content.

Microbial Community Analyses. We used phospholipid fatty acid composition (PLFA) to determine microbial community composition. We extracted 4 glyophilized soil using a modified Bligh and Dyer extraction and identified and quantified individual fatty acids using gas chromatography [60]. Peaks were identified using bacterial fatty acid standards and MIDI peak identification software (MIDI, Inc., Newark, DE, USA). We refer to fatty acids with standard nomenclature as follows: total number of carbon atoms:total number of carbon–carbon double bonds, followed by the position of the double bond from the methyl end of the fatty acid. The suffixes c and t refer to *cis* and *trans* geometry, respectively. The prefixes a and i refer to anteiso- and isobranching. 10Me indicates that a methyl group is attached to the 10th carbon atom from the carboxyl end. Positions of hydroxy groups are indicated with OH. Cyclopropane fatty acids are indicated with *cy*.

Soil Characteristics and Processes. We examined the following soil characteristics in each plot of the study: extractable NH_4^+-N and NO_3^--N, Bray's phosphorus (P), Organic P, moisture, pH, C/N and % C. Subsamples (15 g) of field moist soil were extracted with 100 mL 2N KCl by shaking for 1 h. The extract was passed through Whatman #1 filters, and the filtrate was analyzed for NH_4^+-N and NO_3^--N colorimetrically with an autoanalyzer (Alpkem). We determined available P from air-dried soil using the Bray P1 method [34] followed by colorimetric analysis using an Hitachi U-2001 spectrophotometer. We measured organic P using the Saunders and Williams ignition method [34], again followed by colorimetric analysis. Soil gravimetric moisture content was determined by oven drying 100-g subsamples of field moist soil at 105°C for 48 h. Soil pH was measured on air-dried soil in 0.01 M $CaCl_2$, using a 1:2 (w/v) soil/liquid ratio. Percent C and C/N were determined using a Carlo Erba NA1500 Series II elemental analyzer using the thermal conductivity detector (TCD) with oven-dried soil (60°C).

Statistical Analyses. We compared microbial communities and soil characteristics, in separate analyses, along the plant diversity gradient, among monocultures, and among three-species combinations. Within each of those three comparisons, we also examined whether microbial community composition changed over time or between wet and dry seasons. Microbial community composition comparisons were made using the mole percent of the fatty acids identified in each soil. Fatty acid data were used to generate Bray-Curtis similarity matrices, a measure of community similarity that incorporates the presence as well as relative abundance of individual fatty acids [25, 29]. Specifically, the Bray-Curtis index calculates the similarity between two sites j and k as

$$S_{jk} = \left\{ 1 - \frac{\sum_{i=1}^{p} \left| y_{ij} - y_{ik} \right|}{\sum_{i=1}^{p} \left(y_{ij} + y_{ik} \right)} \right\}$$

where y_{ij} is the mole percent of fatty acid i in site j, y_{ik} is the mole percent of fatty acid i in site k, and p is the total number of fatty acids found in both sites [16]. With these similarity matrices, we performed a nonparametric two-way analysis of similarity (ANOSIM [15]) to test whether there were significant differences in microbial community composition among *a priori* defined treatment groups. To do this, ANOSIM uses the Bray-Curtis similarity matrices to compare ranks of between- to within-group similarities and compares these against a series of random simulations to test the null hypothesis that there are no differences between groups. From the ANOSIM test, the R statistic is calculated. R falls between 0 and 1, and indicates the degree of discrimination between treatment groups. $R = 0$ if the similarities within and between treatment groups are the same, on average, and $R = 1$ if all replicates within treatment groups are more similar to one another than to any replicates from other treatment groups.

We used separate ANOSIM analyses to test the effect of each of the main factors of our study (i.e., plant diver-

sity, monoculture type, or three-species combination type) and included sample date as a second factor in each test. By examining differences across sample dates, we were able to examine changes in microbial composition over time as well as between wet and dry seasons. *P* values of *post hoc* comparisons were Bonferroni adjusted for multiple comparisons [44]. We visualized shifts in community composition between treatment groups and over time using nonmetric multidimensional scaling (MDS) of Bray-Curtis similarity matrices to produce two-dimensional ordination graphs.

To determine the most important fatty acids driving microbial community differences among treatment groups, we used the software SIMPER [16]. The program calculates the average dissimilarity between all pairs of intergroup samples (e.g., it compares each of the monoculture plots to each of the secondary succession plots) and tabulates the average percent contribution of each fatty acid to the dissimilarity observed between the groups.

Total microbial biomass was estimated as the sum of the nanomoles of each of the fatty acid groups present in a given soil. We determined the ratio of bacterial/fungal biomass using the ratio of the relative abundances of the marker fatty acids: i.e., (i15:0 + a15:0 + 15:0 + i16:0 + i16:1 ω7c/i15:0 2OH + 16:1 ω9c + 16:1 ω5c + 10Me 16:0 + i17:0 + a17:0 + cy17:0 + 17:0 + 18:1 ω9c + 18:1 ω7c + cy19:0 ω8c; all bacterial markers)/(18:2 ω6/a18:0; fungal biomarker) [17, 18].

Changes in microbial biomass, bacteria/fungi ratios, and all soil characteristics were analyzed using a repeated measures ANOVA (JMP 4.0). Again, we conducted three separate analyses for each of the main factors of our study (i.e., plant diversity, monoculture type, or three-species combination type). In each of the three analyses, the main factor was treated as a fixed effect and block was treated as a random effect.

To evaluate whether soil characteristics (soil % moisture, extractable NH_4^+ and NO_3^-, Bray's P, % C, C:N, and pH) were related to differences in microbial community composition, we used the statistical software BVSTEP [16]. This program determines if environmental variables, taken either singly or in combination, are related to community composition. To do this, the program first generates a similarity matrix based on each environmental variable and selects the one giving the highest correlation coefficient (ρ) with the matrix for community composition. Each of the remaining abiotic variables is then added, considering all of the pairwise comparisons that include the best single variable, and the best of these pairs is selected. This forward selection is continued until there is no improvement in the correlation coefficient beyond a threshold value (in our case 0.05). To examine potential drivers of microbial community differences across the plant diversity gradient and among plant community types, we standardized (mean 0, standard deviation 1) abiotic variables for each sampling date prior to BVSTEP analysis to remove changes in means across time. To examine potential drivers of microbial community differences across sampling dates, we utilized abiotic data without this standardization. We emphasize that in this analysis, soil characteristics that are at other times presented as means across treatment groups (see Results) are converted to pairwise dissimilarities; thus, characteristics that do not significantly differ among treatment groups in an ANOVA may show a significant relationship to pairwise differences in microbial community composition.

Results

Microbial Variation with Plant Diversity, Plant Community Composition, and Time. Microbial community composition differed significantly among plant diversity

Table 2. Summary of two-way ANOSIM and pairwise *post hoc* test results to determine the effect of plant diversity, monoculture type, and three-species combination on microbial community PLFA composition

	Main treatment		*Sample date*	
Main treatment	*Global R*	*Pairwise comparisons*	*Global R*	*Pairwise comparisons*
Plant diversity	0.231***	1 vs 3*** 1 vs SS** 1 vs 5 ns 3 vs 5 ns 3 vs SS*** 5 vs SS ns	0.625***	All pairwise tests significant
Monocultures	0.379***	*Hyeronima* vs *Cordia*** *Cedrela* vs *Cordia*** *Hyeronima* vs *Cedrela* ns	0.645***	All pairwise tests significant except: Wet 2000 vs dry 2000 ns Wet 2001 vs dry 2002 ns
Three-species combination	0.267***	*Hyeronima* vs *Cordia*** *Cedrela* vs *Cordia* ns *Hyeronima* vs *Cedrela**	0.778***	All pairwise tests significant

Values are significant at $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns = not significant; reported *P* values for *post hoc* comparisons are Bonferroni adjusted. See Table 1 for treatment descriptions.
1 = monocultures, 3 = three-species combinations, 5 = 5-species combinations, and SS = secondary succession plots.

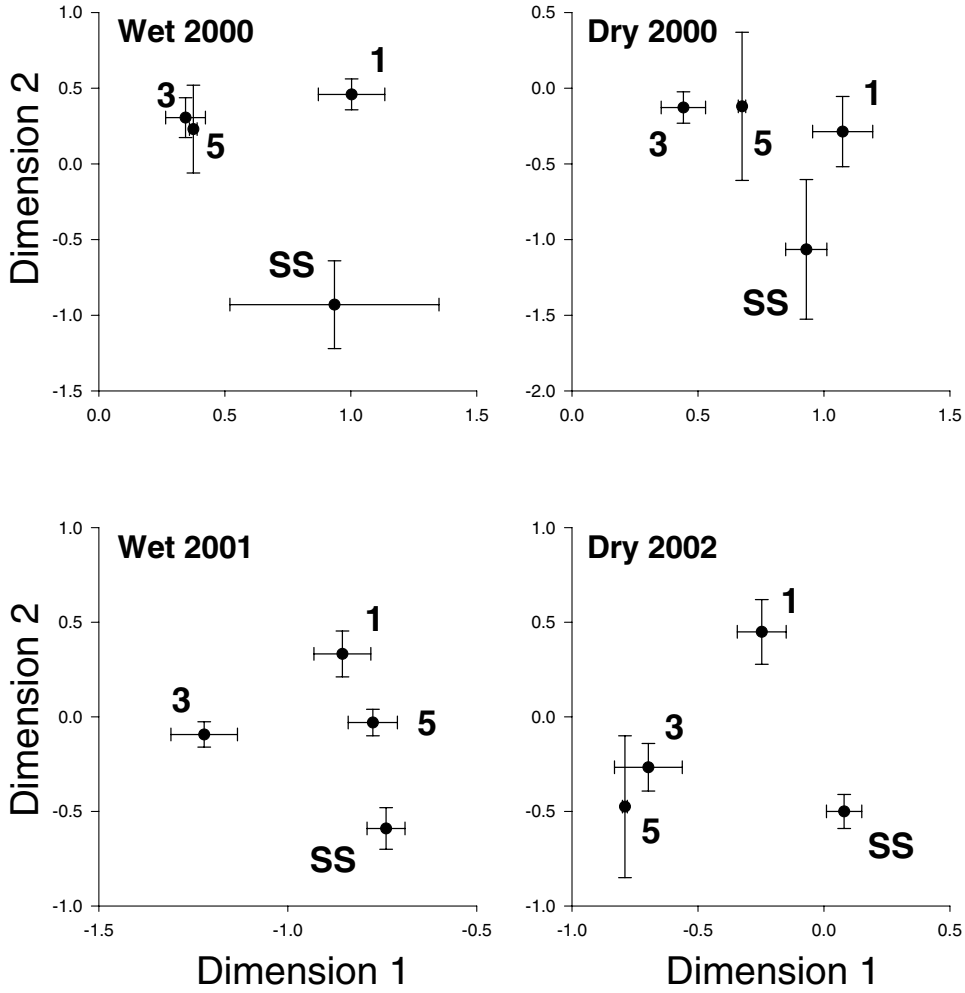

Figure 1. Multidimensional scaling graphs of microbial community composition across a plant diversity gradient for each sampling date. 1 = monocultures, 3 = three-species combinations, 5 = five-species combinations, SS = secondary succession plots. Values are means ± SE. Axes are arbitrary and scaled in units of Bray-Curtis similarity; the closer points are on the graph, the more similar they are in composition. See Table 2 for statistical test results.

Table 3. Soil chemical and microbiological properties across a plant diversity gradient

	Plant diversity level				
Soil property	*1*	*3*	*5*	*SS*	*Significance*
Microbial property					
Biomass (nmol g^{-1})	36.60	47.45	41.92	52.04	***
Bacteria/fungi	84.86	83.42	104.69	60.33	*
Chemical property					
% Moisture	38.0	40.7	40.2	39.1	***
NH_4^+–N (mg kg^{-1})	2.54	2.65	3.17	2.54	ns
NO_3^--N (mg kg^{-1})	1.85	2.40	1.80	2.68	*
Bray's P (mg kg^{-1})	35.76	31.77	40.25	35.39	ns
% C	3.02	3.63	3.30	3.48	*
C/N	11.63	12.27	12.04	12.27	*
pH (in calcium chloride)	5.47	5.55	5.41	5.63	**

P values are from a repeated measures ANOVA; we show the main treatment effect. Values are means across all sample dates. Values are significant at $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, ns = not significant.
1 = monocultures, 3 = three-species combinations, 5 = 5-species combinations, and SS = secondary succession plots.

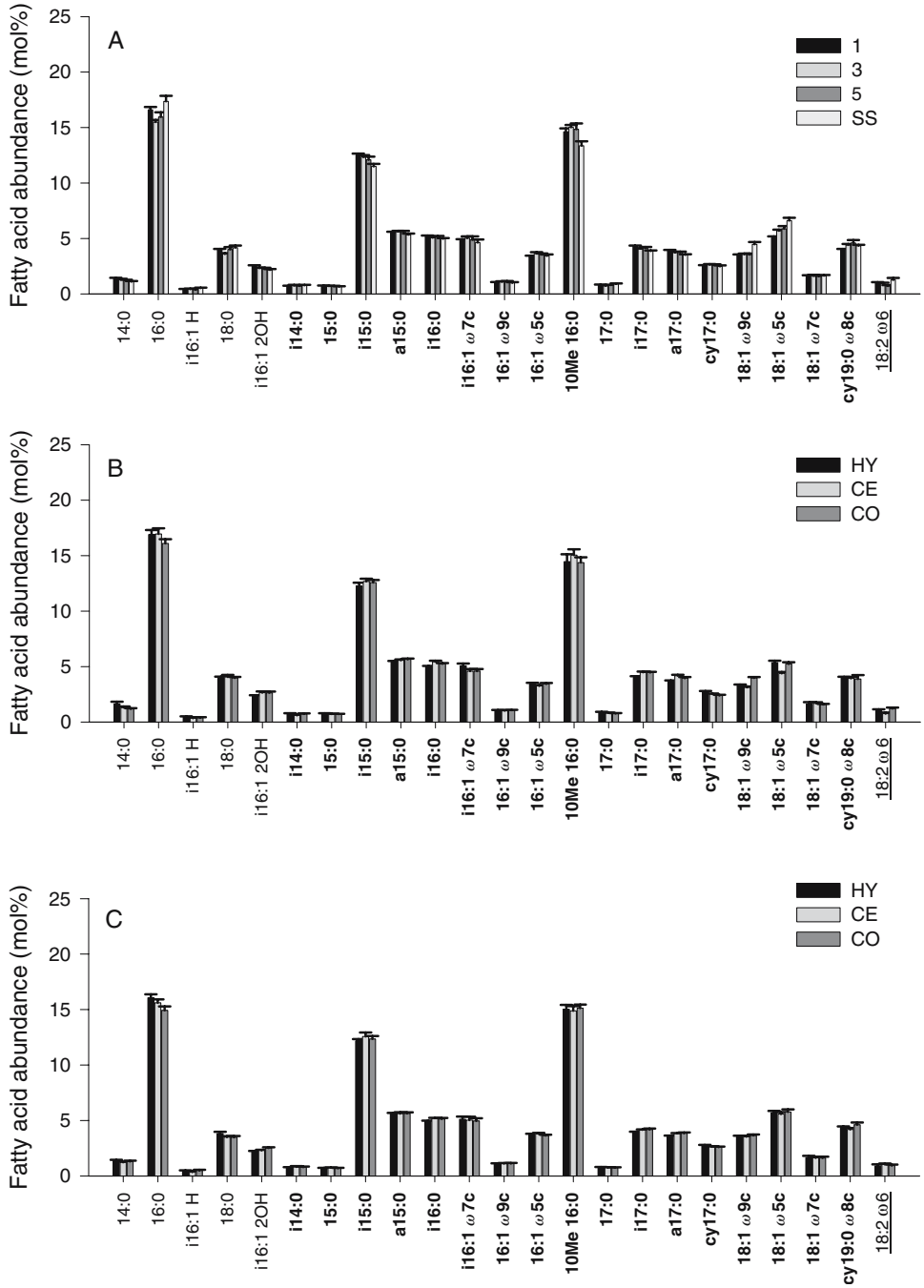

Figure 2. Fatty acid abundances (mol%) across the plant diversity gradient (A), where 1 = monocultures, 3 = three-species combinations, 5 = 5-species combinations, SS = secondary succession plots; among monocultures (B), where HY = *Hyeronima*, CE = *Cedrela*, CO = *Cordia*; among three-species combinations (C) with different focal species, where HY = *Hyeronima*, CE = *Cedrela*, CO = *Cordia*. Bolded fatty acids are those indicative of bacteria, and the fungal fatty acid is underlined (from [17–19, 35, 58]). Values are means across all sampling dates with standard error bars.

levels and among sample dates (Table 2); the effect of sample date was present in all three ANOSIM analyses (Table 2). Because of this effect, we present MDS graphs of PLFA data separately for each sampling date (Fig. 1). *Post hoc* comparisons revealed significant shifts in microbial community composition among monocultures, three-species combinations, and secondary succession plots (Table 2); these differences were apparent in all sampling dates (Fig. 1). Soil microbial community composition in the five-species combinations, on the other hand, did not differ from other treatment groups (Table 2), and shifted relative to other plots at each sampling date (Fig. 1). Microbial biomass and bacteria/fungi varied among plant diversity levels (Table 3). Individual fatty acids varied in

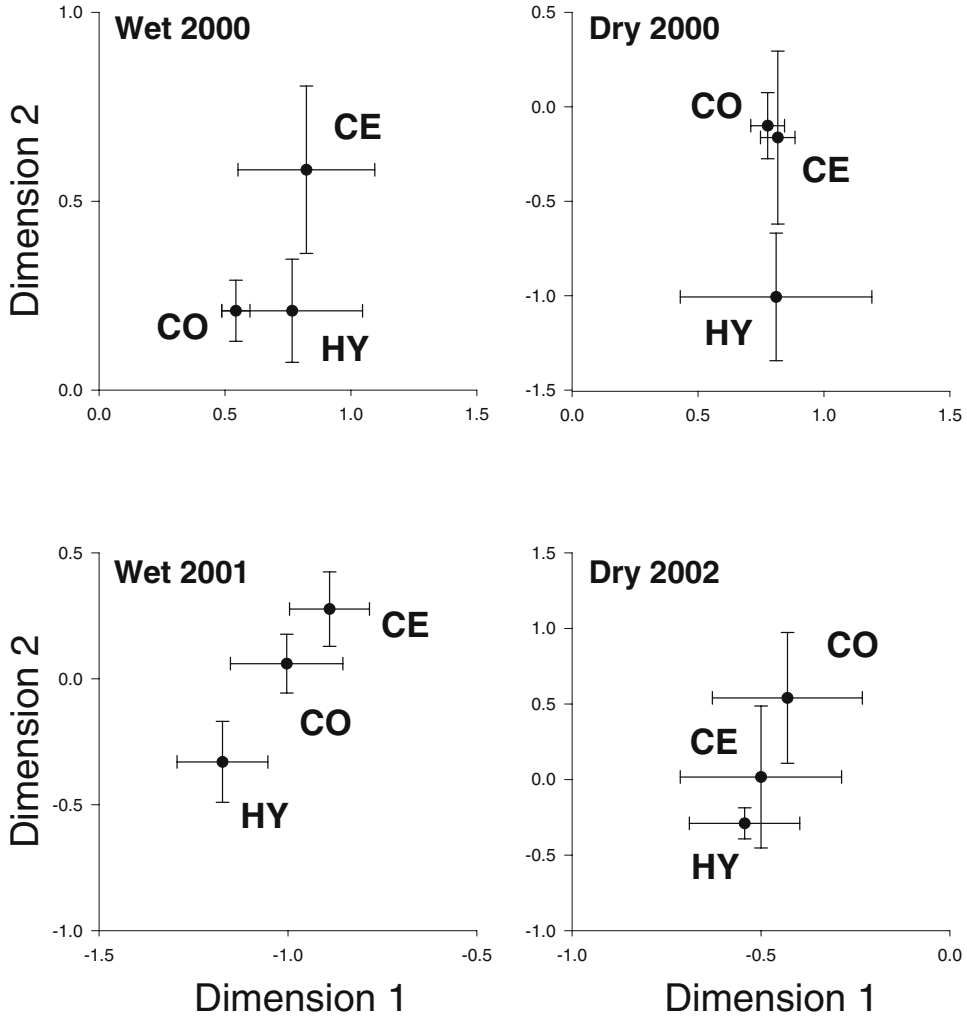

Figure 3. Multidimensional scaling graphs of microbial community composition among monocultures for each sampling date. HY = *Hyeronima*, CE = *Cedrela*, CO = *Cordia*. Values are means + SE. Axes are arbitrary and scaled in units of Bray-Curtis similarity; the closer points are on the graph, the more similar they are in composition. See Table 2 for statistical test results.

Table 4. Soil chemical and microbiological properties among monocultures

	Monocultures			
Soil property	*Hyeronima*	*Cedrela*	*Cordia*	*Significance*
Microbial property				
Biomass (nmol g^{-1})	39.06	32.96	38.01	ns
Bacteria/fungi	85.40	107.03	62.15	*
Chemical property				
% Moisture	40.2	37.8	36.3	***
NH_4^+-N (mg kg^{-1})	2.62	2.25	3.33	ns
NO_3^--N (mg kg^{-1})	1.48	1.78	2.41	*
Bray's P (mg kg^{-1})	38.88	36.25	32.14	ns
% C	3.07	3.09	2.90	ns
C/N	12.84	11.39	10.65	***
pH (in calcium chloride)	5.36	5.51	5.55	**

P values are from a repeated measures ANOVA; we show the main treatment effect. Values are means across all sample dates.
Values are significant at $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns = not significant.

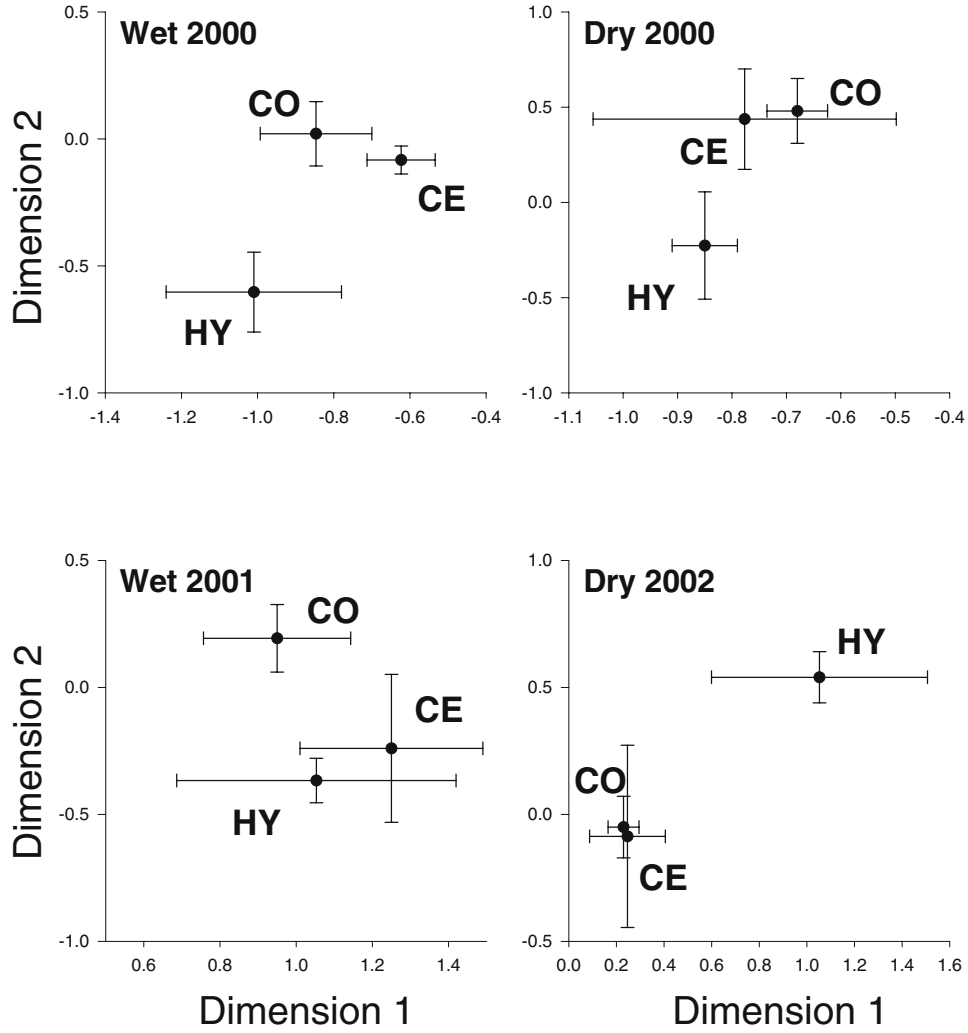

Figure 4. Multidimensional scaling graphs of microbial community composition among three-species combinations with different focal species for each sampling date. HY = three-species plots containing *Hyeronima*, CE = three-species plots containing *Cedrela*, CO = three-species plots containing *Cordia*. Values are means ± SE. Axes are arbitrary and scaled in units of Bray-Curtis similarity; the closer points are on the graph, the more similar they are in composition. See Table 2 for statistical test results.

Table 5. Soil chemical and microbiological properties among three-species combinations

	Three-species type			
Soil property	*Hyeronima*	*Cedrela*	*Cordia*	*Significance*
Microbial property				
Microbial biomass (nmol g^{-1})	46.51	44.32	45.73	ns
Bacteria/fungi	91.82	75.22	83.24	ns
Chemical property				
% Moisture	42.9	39.5	39.7	***
NH_4^+-N (mg kg^{-1})	2.59	2.69	2.67	ns
NO_3^--N (mg kg^{-1})	2.23	1.95	3.07	ns
Bray's P (mg kg^{-1})	33.98	30.23	31.11	ns
% C	3.95	3.29	3.65	**
C/N	12.96	11.99	11.84	***
pH (in calcium chloride)	5.47	5.51	5.67	**

Values presented here are means across all sample dates. *P* values are from a repeated measures ANOVA; we show the main treatment effect. Values are means across all sample dates.
Values are significant at $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, ns = not significant.

Table 6. The three most important fatty acids discriminating microbial composition among plant diversity levels, monocultures, and three-species combinations based on average dissimilarity between all pairs of intergroup samples

Main treatment	*Intergroup comparison*		
Plant diversity	1 vs 3	1 vs SS	3 vs SS
	16:0	10Me 16:0	16:0
	10Me 16:0	16:0	10Me 16:0
	i15:0	18:1 ω7c	i15:0
Monocultures	HY vs CO	CE vs CO	CE vs HY
	10Me 16:0	10Me 16:0	10Me 16:0
	16:0	16:0	16:0
	i15:0	i15:0	i15:0
Three-species combinations	HY vs CO	CE vs CO	CE vs HY
	16:0	16:0	10Me 16:0
	10Me 16:0	10Me 16:0	16:0
	i15:0	i15:0	i16:1 ω7c/i15:0 2OH

For brevity, we show only comparisons among these groups for the plant diversity gradient because five-species combinations were not significantly different from other treatment groups. Bolded columns indicate groups that were significantly different from one another.
1 = monocultures, 3 = three-species combinations, SS = secondary succession plots, HY = *Hyeronima*, CE = *Cedrela*, CO = *Cordia*.

their responses to plant diversity treatments (Fig. 2A); this was true for comparisons among monocultures and three-species combinations as well (Fig. 2B, C).

Within the monoculture plots, microbial community composition significantly varied among species (Table 2). *Post hoc* comparisons revealed that soil microbial communities in *Hyeronima* and *Cordia* monocultures were significantly different (Table 2), and MDS graphs show that these plots clustered separately in all but one sampling date (Wet 2000; Fig. 3). There was also a significant difference between *Cedrela* and *Cordia* monocultures (Table 2), but this result seems to be driven by only one sampling date (Wet 2000; Fig. 3). Bacteria/fungi ratios also varied with monocultures (Table 4), but there was no effect of monoculture type on microbial biomass (Table 4).

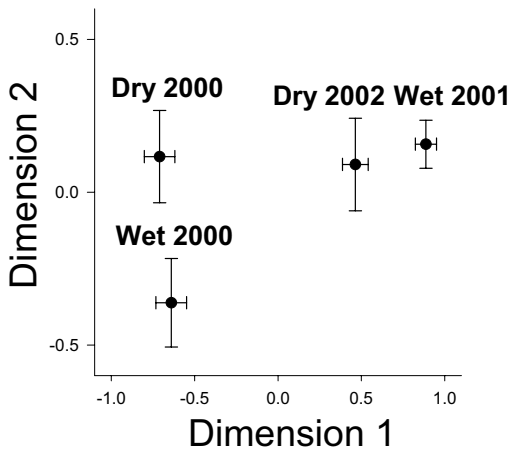

Figure 5. Multidimensional scaling graphs of microbial community composition in all plots across sampling dates. Values are means ± SE (n = 22). Axes are arbitrary and scaled in units of Bray-Curtis similarity; the closer points are on the graph, the more similar they are in composition.

Soil microbial community composition also differed among three-species combinations (Table 2). Pairwise tests revealed that soil microbes in three-species combinations containing *Hyeronima* as the focal species were distinct from those containing *Cordia* (Table 2); this difference was apparent in all sampling dates (Fig. 4). There was also a significant difference between *Hyeronima* and *Cedrela* three-species combinations (Table 2), and this difference was apparent in all but one sampling date (Wet 2001; Fig. 4). There was no effect of three-species type on microbial biomass or bacteria/fungi ratios (Table 5).

The SIMPER analyses revealed that the relative abundance of a few biomarkers (i.e., 10Me 16:0, 16:0, and i15:0) contributed most to the differences in microbial communities between treatment groups (Table 6).

As previously noted, soil microbial community composition changed significantly with sampling date in all plots of this study (Table 2). However, there was no indication that season (wet vs dry) was an important driver of those changes (Fig. 5). For brevity, we show how microbial communities changed over time with one MDS graph of Bray-Curtis similarity values using all plots and sample dates combined rather than showing changes across time individually for each of our focal studies (i.e., the diversity gradient, monoculture, and three-species combinations; Fig. 5).

All the analyses presented here include all fatty acids that were detected and identified in our soils (see Fig. 2). However, a few of these fatty acids are of unknown origin, and are therefore potentially plant-derived (i.e., 14:0, i16:1 H, 16:0, 18:0, and 16:1 2OH). To ensure that the patterns we observed were not driven solely by plant-derived fatty acids, we conducted separate analyses of microbial community composition (ANOSIM) and microbial biomass where these fatty acids were excluded. In no case were the results qualitatively altered by these exclusions. We show only the analyses of the full data set here.

Soil Characteristics. Soil characteristics varied significantly with plant diversity, monoculture type, and three-species combination. Plant diversity differences were reflected in soil moisture, extractable NO_3^-, % C, C:N, and pH (Table 3). Monocultures differed in soil moisture, extractable NO_3^-, % C, C/N, and pH (Table 4). Three-species combinations differed in soil moisture, % C, C:N, and pH (Table 5).

Relating Soil Microbes to Soil Characteristics. The BVSTEP model that was most related to differences in microbial community composition along the plant diversity gradient included only soil C:N ($\rho = 0.052$); this was also true among monocultures ($\rho = 0.078$). Among three-species combinations, Bray's P was the only factor related to differences in microbial community composition ($\rho = 0.085$). Across sample dates, the BVSTEP model most related to changes in microbial communities included soil C:N and Bray's P ($\rho = 0.155$); however, Bray's P accounts for most of the variability captured in the model ($\rho = 0.147$ for Bray's P alone).

Discussion

Our study showed that soil microbial community composition varied with differences in tropical plant diversity and composition. Along the plant diversity gradient, the largest differences in microbial community composition were among monocultures, three-species combinations, and secondary succession plots (Fig. 1). Microbial community composition also varied among monoculture types, among three-species combinations with different focal species (Figs. 3 and 4, respectively), and among sampling dates (Fig. 5).

Despite intensified interest in understanding the link between above- and belowground biodiversity and the implications that this link may have for ecosystem functioning [2, 11, 23, 40], few studies have examined the relationship between plant diversity and soil microbial communities using culture-independent techniques; the results from the few that have are equivocal [8, 9, 58, 61]. For example, Broughton and Gross [9] examined microbial fatty acid methyl esters (FAMEs) along a topographic gradient in a mid-successional temperate grassland that varied in plant diversity (from 2 to 16 species) and productivity. They found no relationship between FAME composition and either factor, but they did find that microbial activity was positively correlated to productivity. Brodie *et al.* [8] used analysis of 16S rDNA to demonstrate that microbial genetic diversity increased with decreasing plant diversity across a grassland transect in Ireland; the increase in microbial diversity was correlated with increases in soil organic matter and microbial biomass. Zak *et al.* [61] found that the abundance of different PLFAs varied among plant diversity levels in a long-term experimental grassland system, and that these differences were linked to the plant productivity. Wardle [58] observed no effect of plant diversity on microbial fatty acid diversity or abundance in a glasshouse experiment that examined the response of multiple trophic levels to plant diversity and composition; however, they did observe strong effects of plant species identity on fatty acid diversity and abundance. Our study, which demonstrates a strong relationship between fatty acid composition and plant diversity, is the first such study to be done in the tropics, where anthropogenic reductions of biodiversity are most intensified and yet where the functional implications of such losses have been least studied.

Given the lack of consensus of these studies, it is difficult to derive any wide-ranging conclusions about the influence of plant diversity on soil microbial communities. However, it is likely that the use of different methods to detect soil microbes (i.e., FAME, PLFA, DNA) contributed to the inconsistent results. For example, the Broughton and Gross [9] study used FAME analysis, which incorporates fatty acids from detritus as well as soil microorganisms and is less sensitive than PLFA analysis in detecting shifts in microbial communities [36]. The other fatty-acid-based studies (i.e., [58, 61], and this study) all used PLFA analysis, but the results from these studies also varied. It is possible, as suggested by Wardle [59] that plant diversity exerts a detectable effect on soil microbial communities only when plant diversity increases plant productivity. This finding is supported by Zak *et al.* [61], where plant diversity was associated with increases in productivity, as well as Brodie *et al.* [8], where microbial diversity was positively related to soil organic matter concentrations. We did not have productivity estimates for the secondary succession sites in our study, but we observed an increase in soil carbon concentrations with plant diversity, which may be a reasonable index of plant productivity [39]; thus, our study also supports the Wardle [59] hypothesis. Clearly, we are just beginning to understand the link between plant diversity and soil microbial community composition, but it is clear that the methods used to detect microbial community change and the range of plant productivity included in the study system are both likely to influence the nature of that relationship.

As with studies attempting to relate species diversity to ecosystem function, we cannot attribute the changes we observed in microbial communities along the diversity gradient to the number of plant species *per se* [24, 26, 48, 50]. The likelihood that a study site contained a species that had a strong effect on microbial community composition increased with plant diversity in this study (i.e., the sampling effect [57]). Our observations that microbial community composition differed among plots that harbored distinct plant communities with the same number of species suggests that this effect could have influenced the patterns we observed. Put simply, plant species identity

may be as likely as the number of plant species to have influenced the composition of soil microbial communities in this system (see [4, 30]).

Of all the soil characteristics we examined, we expected that the variability in microbial community composition would be best explained by differences in soil carbon quantity and quality. In fact, soil C/N, an indicator of the relative quality of soil organic matter [41], was the abiotic variable most related to differences in microbial community composition across the plant diversity gradient and among monocultures. We expected soil carbon concentrations to play an important role as well, given the importance of available energy in structuring communities [8, 38], but we found no evidence of such a link. Although we did find a correlation between microbial community differences and soil C/N, it was always low (the correlation coefficient never exceeded 0.10). These low correlations suggest that plant-community-driven differences in soil microbial communities may not have been mediated via soil characteristics; the small differences in soil characteristics among treatments support this suggestion (Tables 3, 4, and 5). It is also possible that microbes were affected by soil characteristics that we did not measure in this study (e.g., dissolved organic carbon concentrations) or were directly influenced by plant traits (e.g., root exudation, turnover, or density).

In addition to differences across plant treatments, we also expected to find significant shifts in microbial communities between wet and dry seasons, given previous studies that have shown changes in PLFA profiles within and among seasons [6, 42, 43, 45]. Instead, we observed shifts that were independent of season and showed no consistent trend over time (Fig. 5, Table 2). In fact, microbial community composition was more strongly affected by sampling date than plant diversity or composition (Table 2). Although this was in part due to having higher statistical power when testing among sampling dates, it was clearly an important factor structuring microbial communities. It is possible that a more fine-scaled temporal sampling during wet and dry seasons would have revealed consistent shifts between seasons. However, we observed no significant effect of sampling date on soil moisture (repeated measures ANOVA, $F_{3,70} = 2.23$; $P > 0.05$), which remained between 38% and 41% in all sampling dates; apparently, there was sufficient rainfall in the dry season to maintain soil moisture at a near-constant level. Therefore, soil microorganisms in this relatively wet system may be responding more strongly to variables other than rainfall that change with time and are independent of season (e.g., pulses of litter or root production). This is supported by our finding that differences in microbial community composition over time were not related to changes in soil moisture.

Our findings provide evidence for human alteration of soil microbial communities via the alteration of plant diversity and/or composition. Given the critical role that microbes play in ecosystem processes, it is possible that these modifications in soil microbial communities may have implications for ecosystem functioning. In fact, a few recent studies have related microbial community composition to rates of microbially mediated processes (i.e., nitrification, denitrification, decomposition, and enzyme activity; [10, 12–14, 56]), but this relationship is not always evident [37]. It may be particularly important to understand these dynamics in the tropics, where the majority of the world's biodiversity and productivity resides.

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