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A comparison of nutrient dynamics and microbial community characteristics across seasons and soil types in two different old growth forests in Costa Rica

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Abstract: The use of nutrient and microbial metrics were examined as potential indicators of change in microbial community structure and nutrient dynamics across landscape-scale nutrient and seasonal moisture gradients in the Carbono Project forest soils in Costa Rica. Previous studies showed a decrease in net primary productivity occurred in these forests during the warmer years. In this study, differences were found in soil microbial community structure and function and nutrient dynamics across seasons and soil types. Most critically, when the soil moisture decreased in the dry season, there was a significant decrease in fungal contribution, carbon biomass development, and efficiency of carbon utilization in the soils. The implications are that should these forests continue to become warmer or the dry season lengthen, it could have measurable impacts on the soil biomass development and soil ecosystem health. The metrics from this study appear to have potential use for monitoring forest soil conditions.

Resumen: Se examinó el uso de métricas de nutrientes y microbianas como indicadores potenciales de cambio en la estructura de la comunidad microbiana y la dinámica de nutrientes a través de gradientes de escala paisajística de nutrientes y de humedad estacional en los suelos forestales del Proyecto Carbono en Costa Rica. Estudios previos mostraron que hubo un decremento en la productividad primaria neta en estos bosques durante los años más cálidos. En este estudio se encontraron diferencias en la estructura y la función de la comunidad microbiana del suelo y en la dinámica de nutrientes entre estaciones y tipos de suelo. Lo que es aún más crítico, cuando decreció la humedad del suelo en la estación seca hubo un decremento significativo en la contribución fúngica, el desarrollo de biomasa de carbono y la eficiencia de utilización de carbono en los suelos. Esto implica que si estos bosques continúan tornándose más cálidos o si se alarga la estación seca, podría haber impactos medibles en el desarrollo de la biomasa del suelo y en la salud del ecosistema edáfico. Las métricas de este estudio parecen tener un uso potencial para el monitoreo de las condiciones del suelo del bosque.

Resumo: No âmbito do Projecto Carbono nos solos florestais na Costa Rica, o uso de nutrientes e as métricas microbianas foram examinadas como indicadores potenciais da mudança de estrutura da comunidade microbiana e da dinâmica de nutrientes em toda a escala de paisagem quanto aos nutrientes e gradientes sazonais de humidade nos respectivos solos.

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Estudos anteriores mostraram que uma diminuição na produtividade primária líquida ocorreu nestas florestas durante os anos mais quentes. Neste estudo, encontraram-se diferenças na estrutura da comunidade microbiana do solo, e na função e dinâmica de nutrientes através das estações e tipos de solos. De forma mais críticaverificou-se que quando a humidade do solo diminuiu na época seca, houve uma diminuição significativa na contribuição dos fungos, no desenvolvimento da biomassa de carbono e na eficiência da utilização de carbono nos solos. As implicações são de que se estas florestas continuarema tornar-se mais quentes, ou a estação seca mais longa, poderá ocorrer um impacto mensurável sobre o desenvolvimento da biomassa do solo e a saúde do respectivo ecossistema. Os valores decorrentes deste estudo parecem ter um uso potencial para monitorizar as condições do solo da floresta..

Key words: Biomass estimates, carbon cycling, fungi, microbial community, nitrogen, organic matter, phosphorus, rRNA abundance, laccase gene, nifH gene.

Introduction

The Carbono Project, implemented in 1997 at the La Selva Biological Station in Costa Rica, has been a long-term effort to study the processes involved in forest carbon (C) cycling in an old-growth tropical rain forest, and how these processes change across landscapes, over time, and with climatic variation (Espeleta & Clark 2007). Work from this project identified that annual aboveground forest productivity was strongly reduced during periods of higher night time temperatures over a recent 16-yr period, and was also negatively correlated with the estimated pan-tropical terrestrial CO_2 flux during the same period (Clark *et al.* 2003). It is not clear how such environmental changes impact the soil microbial structure and function in these forests.

There is ample evidence that certain environmental changes will impact the soil microbiota. For example, elevated levels of CO_2 affect soil microbial community structure and function, and the dynamics of the C, phosphorus (P) and nitrogen (N) nutrient cycles (Anderson 2003; Bradford *et al.* 2008; Cleveland *et al.* 2004; Pendall *et al.* 2008; Zak *et al.* 2000). Increasing temperatures affect various belowground processes including productivity (Litton & Giardina 2008; Pendall *et al.* 2008). Changes in soil moisture alter soil microbial activity and associated nutrient cycle processes (Campo *et al.* 2001; Eaton 2001; Luizao *et al.* 1992; Schwendenmann & Veldkamp 2006; Zhang & Zak 1995).

As soil abiotic and biotic structure and function are key in nutrient cycle dynamics and plant community composition (Fierer *et al.* 2007; Kent & Triplett 2002; Litton & Giardina 2008; Wardle 2006), physiologic and diversity measurements are being used as indicators of soil ecosystem condition (e.g., Anderson 2003; Hartmann & Widmer 2006; He *et al.* 2003; Ibekwe *et al.* 2007; Schmidt 2006; Torsvik & Øvereås 2002; Wardle 2006). In particular, are the indicators of organic matter decomposition and utilization, CO_2 flux and processing, the N and P cycle processes, microbial biomass, relative contribution of fungi and bacteria, and the presence of critical microbial taxa (Buckley & Schmidt 2003; He *et al.* 2003; Moscatelli *et al.* 2005; Priess & Fölster 2001).

The goal of this study was to determine if a suite of nutrient and microbial metrics could be used as indicators of change in microbial community structure across landscape-scale nutrient gradients and between the dry and wet seasons in the Carbono Project forest soils. Soil was analyzed for community-level diversity and relative contribution of fungal and bacterial rRNA and the functional genes nifH (for N-fixation) and laccase (for lignin degradation), microbial biomass C, efficiency of microbial utilization of organic C, and levels of P and inorganic N. These were examined as potential indicators of soil habitat condition to explore three hypotheses: (1) microbial community structure differs between old-growth stands on the younger (nutrient rich) and older (nutrient poor) oxisols underlying La Selva (Espeleta & Clark 2007); (2) microbial composition changes between the wet and dry season; and (3) less soil microbial biomass exists and less microbial activity occurs during the dry season.

Materials and methods

Sample sites and soil collection

The old growth forests studied are part of the La Selva Biological Station in Costa Rica (10°26' N, 84°00' W), which is operated by the Organization for Tropical Studies (OTS), and have been previously characterized as "Tropical Wet Forest" (Hartshorn & Hammel 1994), with a mean annual rainfall of about 4300 mm, and mean annual temperature of ca. 25 °C (OTS records). Soil samples were collected in the wet season (April) and dry season (July) of 2008, from nine of the 18 previously described 0.5 ha Carbono Project plots (Espeleta & Clark 2007); five plots were from the younger oxisol or "A" soil types, and four from the older oxisol or "L" soil types soils. The younger oxisol soils are terraces of alluvial deposition origin, and are deeply weathered, with yellowish-brown clay and low base saturation. They are considered Typic Haploperox soils. The older oxisol soils are of basaltic lava flow origin, with reddish-brown clay, and also with low base saturation, and are also classified as Typic Haploperox soils. Both soil types have been found to have pH values of about 4.4 to 5 and organic C levels of about 4.0 to 5.5 %. The main differences between the A and L type soils are that the L soil types historically have been found with slightly higher basal respiration rates, slightly greater organic C levels, and lower P levels (Espeleta & Clark 2007; Schwendenmann & Veldkamp 2006; Wood *et al.* 2005). One 2-cm diameter soil core was extracted to 10 cm depth at 16 regularly spaced locations throughout each 0.5 ha plot. Soils were composited by plot, sieved while field moist, and subsampled for subsequent analysis. The percent water content was determined for each sample by weighing 10 g of soil before and after incubation at 105 °C.

Phosphorus and inorganic nitrogen analyses

Phosphorus in the form of phosphate was extracted from 4 g of fresh soil with 30 ml 0.5 M $NaHCO_3$ solution immediately upon collection as per McGroddy *et al.* (2004). Solutions were frozen until analysis. Concentrations of P in the bicarbonate extract solutions were determined using molybdate blue colorimetric reactions measured on an Alpkem Flow Solution IV 250 Auto Analyzer (OI Analytical; College Station, Texas, USA) at the University of Virginia. The $NaHCO_3$ solution is thought to extract P loosely bound to soil surfaces as well as some P associated with the microbial pools and is considered readily available for uptake by both plants and microbes (Cross & Schlesinger 1995).

Inorganic N was determined as the amounts of ammonium (NH_4-N), nitrate (NO_3-N), and combined values as total mineral nitrogen (TMN) in the soils following 2M KCl extraction (Keeney & Nelson 1987) from 10 g soil samples from each of the nine soil types. Extracts were frozen for later analysis at University of Virginia. The N extracts were analyzed colorimetrically on a Lachat QuikChem 8500 (Hach Co., Loveland, CO). The chemical analysis was complicated by iron-rich extracts. Therefore, prior to running the extracts, we added citrate to the extracts to chelate iron (Rhine *et al.* 1998).

Microbial biomass analysis

The microbial biomass C (Cmic) was determined for each sample by the fumigation-extraction method (Jenkinson 1988). Briefly, this is the difference between the extractable (in 50 ml K_2SO_4) soil organic C (SOC) levels in ethanol-free chloroform-fumigated and unfumigated 10 g soil subsamples. The SOC levels were determined by dry combustion analysis at the CATIE labs in Turrialba, Costa Rica, using the methods of Anderson & Ingram (1993) and an autoanalyzer (Alliance Instruments). The ratio of Cmic:SOC was used as an indicator of microbial efficiency of utilization of SOC, and is suggestive of the availability of carbon to the system in soil (Anderson 2003; He *et al.* 2003; Moscatelli *et al.* 2005).

Microbial and functional gene abundance analysis

The optimum number of cycles needed to generate the maximum amount of target DNA while still in log increasing phase was predetermined for the different PCR assays using known concentrations of purified target DNA and the different sets of primers. For the PCR assays, the DNA sequences of the universal bacterial rRNA primers 27f/1492r, the universal fungal rRNA primers EF4/Fung5, the N-fixation gene primers nifHf/nifHr, and the laccase gene primers lacIf/lacII, the reaction conditions and the temperature schedules of Martin-Laurent *et al.* (2001), Smit *et al.* (1999), Minerdi *et al.* (2001), and D'Souza *et al.* (1996) were used for the project PCR assays, along with and the pre-determined number of PCR cycles of 30, 25, 25, and 30 cycles, respectively.

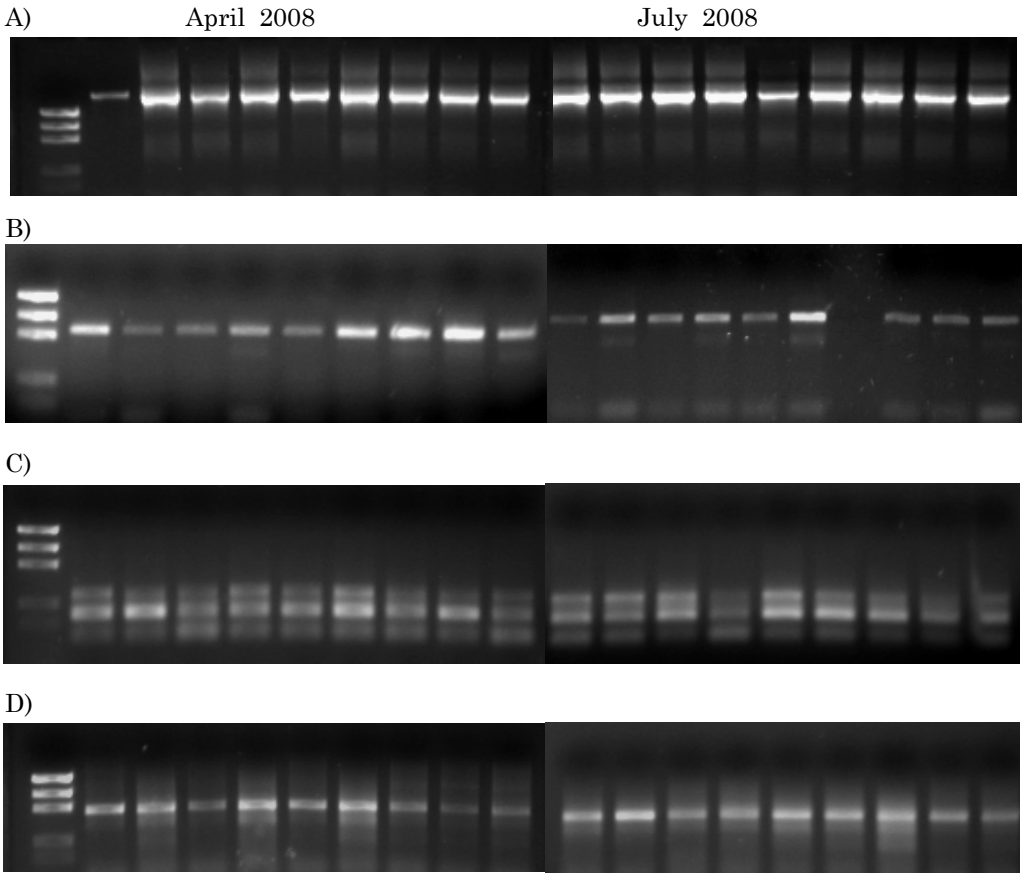


Fig. 1. Agarose gel analyses of PCR products using soil DNA and four different PCR primers: (A) the 27f/1492r primers generate a DNA fragment approximately 1300-1400 bp; (B) the EF4/Fung5 primers generate a fragment approximately 550-600 bp; (C) the lac1/lacII primers generate 2 fragments at about 140 and 240 bp; and (D) the nifHf/nifHr primers generate fragments about 490 bp. Gels are from DNA extracted from soil in April and July, 2008.

Replicate DNA extractions were performed from each of the nine composited soil samples using the Power Soil DNA extraction kit from MoBio, Inc (Carlsbad, CA). The soil DNA concentration was determined by agarose gel electrophoresis, using the Bio Rad Precision Molecular Marker Mass Standard and Gene Tools software to determine DNA concentrations. The average final concentration of DNA from the April soils was 40 $\mu g\ \mu L^{-1}$ and the July soils 38.6 $\mu g\ \mu L^{-1}$. Replicate PCR assays were performed on 40 ng of the soil DNA samples using the different PCR primers and the reaction conditions from above. The replicate PCR products from an individual soil sample were combined and the DNA concentrations determined as DNA band signal intensity of PCR products per ng of soil DNA used. Fig. 1 shows the agarose gel analyses of the PCR products, demonstrating the size of the bands as consistent with that previously described : 27f/1492r primers generate a fragment about 1300 bp (Martin-Laurent *et al.* 2001); the EF4/Fung5 primers generate a fragment about 550 bp (Smit *et al.* 1999); the lac1/lacII primers generate 2 fragments at about 140 and 240 bp (D'Souza *et al.* 1996); and the nifHf/nifHr generates fragments about 490 bp (Minerdi *et al.* 2001). The DNA quantities were compared between gels and profiles by proportionally adjusting the DNA quantity given in a gel by the ratio of ng of mass marker DNA in that gel to the maximum ng of mass marker DNA in the comparative series (Dunbar *et al.* 2001).

To compensate for the possible presence of PCR reaction inhibitors in a DNA sample, a Relative Percent Contribution (RPC) value was determined for each gene assessed, rather than using

the measured ng of the PCR product. This provides an estimate of the level of contribution that the target PCR gene product represents with respect to all PCR values from that one sample. The RPC values were determined by dividing the ng of a PCR product by the sum of the ng of all PCR products from that one DNA sample. For example, to determine the RPC of nifH gene in a single DNA sample:

RPC nifH = [(ng nifH) ÷ (ng nifH+ ng laccase+ ng bacterial rRNA+ ng fungal rRNA)]

These RPC values do not suggest an actual amount of the marker gene in the samples. Rather, they provide a preliminary estimate of the relative percent contribution that each gene might be making in an individual soil DNA sample, with respect to the other marker genes. These levels of contribution can then be confirmed with more detailed Real - Time PCR studies in the future. This method was also used to determine the relative ratio of fungal to bacterial rRNA (fn:fn+bc) that was used to estimate if soils were bacterial or fungal-dominated.

Microbial diversity analyses

The relative diversity of the microbial community was assessed using restriction fragment length polymorphism (RFLP) analysis of 20 µL of bacterial and fungal community rRNA PCR-amplified products digested with either 5U of Hha I or 5U Hha I combined with 5U Hind III enzymes, respectively, and incubated at 28 °C overnight to allow digestion to occur. The sample DNA was separated by 2 % agarose gel electrophoresis, and the RFLP DNA banding patterns determined for each of the 9 samples. The size (as base pairs) and mass (ng) of each band were identified using Gene-Tools software.

Gene Tools software allows clear separation of DNA fragments of several bp difference. However, to be conservative in our diversity estimates, we considered DNA fragments < 15 bp different to be a single size fragment type, using the mean number of bp in the fragment group as the final size type. A consensus table was developed of the RFLP-based DNA bands present for the two different soil types and by season, and the mean values of the size and mass of the bands recorded. Comparisons were also made in the difference in the number of distinct bands between the dry and wet season A and L soil type samples, the dry plus wet season A and L samples. All data were then used to determine RFLP-based Shannon-Weiner diversity index (Shannon & Weaver 1963) by habitat type and by season.

Statistical analyses

For microbial community structure profiling in this preliminary study, analyses of the mean differences in the soil parameters was conducted using multiple methods that are recommended for analysis of small sample sizes. In addition to the use of 2-sample randomization t-tests, the percent difference (PD) and Cohen's *d* (Cohen 1977) were determined. The PD between mean values were used to suggest the magnitude of the difference, and the Cohen's *d* values to suggest the potential for a meaningful difference between two means. Due to the small sample sizes in this preliminary study there was a possibility for large variability between measured values. To account for this, a weight of evidence approach was used in which mean differences with P values ≤ 0.1 and d values ≥ 0.7 (0.8 is considered "a large mean effect size difference"; Cohen 1977), or P values ≤ 0.2, d values ≥ 0.7, and PD values ≥ 14 % were considered to represent meaningful differences between two mean values. Correlations were made between the different parameters and Cmic and Cmic:SOC, RPC of bacterial and fungal rRNA genes, and the RPC of the nifH and laccase genes. The r values at $P = 0.1$ that were ≥ 0.2 or ≤ -0.2 were considered to represent critical correlations. All calculations were made using R (Free Software, version 2.9) or NCSS (version 2007; NCSS Kaysville, Utah) statistical software.

The data were also examined for the relative extent of the relationships between different habitat types and seasons by cluster analysis using a similarity matrix and Ward's minimum variance method of measuring Euclidean distances (Hartmann & Widmer 2006) in NCSS software. The relative abundances of the RFLP-based DNA fragments were also compared by habitat type and by season, along with the H values, by cluster analysis in NCSS.

Results

Nutrient and microbial gene abundance analysis

Mean differences in the various parameters were found between samples by both soil type and season that met the criteria to suggest meaningful biological differences (Tables 1 and 2). Inorganic N and Cmic values were greater and the P levels less

Table 1. Mean values (standard deviation) of microbial biomass carbon (Cmic), microbial efficiency of organic carbon utilization (Cmic:SOC), ammonium (NH_4-N), nitrate (NO_3-N), phosphorus (P), percent soil moisture (% H_20), and the relative percent contribution (RPC) of the nifH, laccase(lac), bacterial rRNA (bact), and fungal rRNA (fungi), and the ratio of fungal rRNA to fungal rRNA plus bacterial rRNA (fn:fn+bc) by A (younger oxisols) and L (older oxisols) soil types and by dry and wet season.

Soil Type	H_20 (%)	NH_4-N ($\mu g\ g^{-1}$)	NO_3-N ($\mu g\ g^{-1}$)	TMN ($\mu g\ g^{-1}$)	P ($\mu g\ g^{-1}$)	Cmic ($mg\ g^{-1}$)	Cmic : SOC
Type A Soil	42.0 (3.0)	1.8 (1.0)	30.2 (9.0)	32.0 (8.9)	7.7 (2.7)	0.198 (0.038)	5.2 (1.2)
Type L Soil	41.0 (3.4)	4.6 (6.6)	42.4 (15.1)	47.0 (20.7)	5.3 (1.4)	0.244 (0.043)	6.0 (1.3)
Dry Season Soil	36.5 (2.3)	5.1 (5.8)	38.2 (18.6)	43.3 (22.8)	7.6 (1.5)	0.202 (0.050)	4.8 (0.8)
Wet Season Soil	45.9 (1.4)	1.0 (0.43)	32.6 (4.4)	33.6 (4.4)	5.7 (3.0)	0.235 (0.036)	6.4 (0.7)
	RPC nifH	RPC lac	RPC bact	RPC fungi	RPC fn:fn+bc		
Type A Soil	28.0 (8.7)	32.7 (13.0)	20.8 (6.7)	18.5 (5.4)	47.7 (10.3)		
Type L Soil	19.2 (5.4)	32.8 (9.4)	20.2 (6.2)	27.8 (10.4)	56.6 (16.2)		
Dry Season Soil	26.2 (10.9)	33.0 (15.9)	22.6 (7.1)	18.1 (5.4)	45.3 (9.4)		
Wet Season Soil	21.9 (9.9)	32.4 (4.1)	18.5 (4.9)	27.1 (10.1)	58.1 (14.6)		

Table 2. The percent differences (PD), t-test *P* and effect size *d* values for comparing the percent water moisture (% H_20), microbial biomass C (Cmic), microbial efficiency of organic C utilization (Cmic:SOC), ammonium (NH_4), nitrate (NO_3), total mineralized N (TMN), phosphorus (P), relative percent contribution (RPC) of the nifH, laccase (lac), bacterial (bact) and fungal rRNA (fungi), and the ratio of fungal to fungal and bacterial rRNA (fn:fn+bc) in A (younger oxisols) and L (older oxisols) soil types and by season (ns = not significantly different).

Comparison	% H_20	Cmic	Cmic : SOC	NH_4-N	NO_3-N	TMN	P
L to A Soil Type							
PD	-3.30	18.90	12.50	60.50	28.70	31.80	-30.70
P values	ns	0.192	ns	0.198	0.054	0.058	0.041
d values	ns	1.2	ns	0.7	1.1	1.1	1.1
Wet to Dry Season							
PD	9.40	14.00	24.60	-80.40	-14.60	-22.40	-24.50
P values	0.0001	0.128	0.004	0.049	ns	0.2	0.117
d values	2.8	0.8	2.9	1.1	ns	0.7	0.8
Comparisons	RPC nifH	RPC Bact		RPC lac	RPC fungi	RPC fn:fn+bc	
L to A Soil Type							
PD	-31.40	-2.90		0.30	33.40	15.70	
P values	0.024	ns		ns	0.026	0.173	
d values	1.3	ns		ns	1.24	0.7	
Wet to Dry Season							
PD	-16.40	-18.10		1.80	33.20	22.00	
P values	ns	0.172		ns	0.03	0.043	
d values	ns	0.7		ns	1.2	1.1	

Table 3. Correlation analysis of soil data. The *r* values at $P = 0.1$ that were ≥ 0.2 or ≤ -0.2 were considered to represent critical correlations (identified in bold). The data are combined from the values measured from the A (younger oxisols) and L (older oxisols) soil types collected in April and July from within the Carbono Project forest plots in La Selva, Costa Rica.

	nifH	Fungi	Laccase	Bacteria	Laccase to nifH	Fungi to Bacteria	% Water	Cmic	Cmic to SOC	P	NH_4	NO_3	TMN
nifH	1	0.012	-0.81	0.1	-0.89	-0.14	-0.1	-0.39	-0.33	0.48	-0.31	-0.2	-0.25
Fungi	0.012	1	-0.15	-0.17	-0.37	0.69	0.15	0.39	0.36	-0.23	-0.31	0.07	-0.02
Laccase	-0.81	-0.15	1	-0.44	0.89	0.26	0.31	0.21	0.31	-0.42	0.11	-0.21	-0.14
Bacteria	0.1	-0.17	-0.44	1	-0.29	-0.81	-0.3	0.18	0	0.23	0.17	0.36	0.33
Laccase to nifH	-0.89	-0.37	0.89	-0.29	1	0.06	0.14	0.14	0.16	-0.39	0.31	0.01	0.09
RPC fn:fn+bc	-0.14	0.69	0.25	-0.81	0.06	1	0.27	0.13	0.21	-0.33	-0.32	-0.2	-0.25
% Water	-0.1	0.15	0.31	-0.3	0.14	0.27	1	0.23	0.61	-0.27	-0.44	-0.14	-0.23
Cmic	-0.39	0.39	0.21	0.18	0.14	0.13	0.23	1	0.89	-0.27	-0.15	0.32	0.22
Cmic to SOC	-0.33	0.36	0.31	0	0.16	0.21	0.61	0.89	1	-0.37	-0.31	0.13	0.02
P	0.48	-0.23	-0.43	0.23	-0.39	-0.33	-0.27	-0.28	-0.37	1	-0.05	-0.08	0.08
NH_4	-0.31	-0.31	0.11	0.17	0.31	-0.32	-0.44	-0.15	-0.31	-0.05	1	0.65	0.79
NO_3	-0.2	0.07	-0.21	0.36	0.01	-0.2	-0.14	0.32	0.13	-0.08	0.65	1	0.98
TMN	-0.25	-0.02	-0.14	0.33	0.09	-0.25	-0.23	0.22	0.02	-0.08	0.79	0.9	1

in the L soils as compared to the A soils. The RPC of nifH gene was less and RPC of the fungal rRNA gene and the RPC fn:fn+bc ratio was greater in the L than the A soils. Soil samples from the wet season had a greater percent water content, Cmic, Cmic:SOC ratio, and RPC of fungal rRNA gene and RPC fn:fn+bc ratio, but less P, NH_4-N , NO_3-N, TMN, and RPC bacterial rRNA gene when compared to the dry season samples. Cluster analysis of the data suggested a closer relationship existed between the soils by season than by plot type (Fig. 2).

The correlation analyses suggested a variety of possible relationships that warrant further study (Table 3). In particular, soil moisture was positively correlated with Cmic, the ratios of Cmic to SOC and fungal to bacterial rRNA, and laccase, and negatively correlated with bacterial RPC, P and inorganic N. The biomass was positively correlated with fungal and laccase RPC, inorganic N, and water, and negatively correlated with nifH gene RPC and P. Inorganic N was positively correlated with bacterial RPC and negatively correlated with nifH gene RPC. The nifH gene RPC was positively correlated with P and negatively correlated with laccase gene RPC, biomass, and all three measurements of inorganic N. The P was positively correlated with bacterial and nifH gene RPC and negatively correlated with biomass, fungal and laccase RPC.

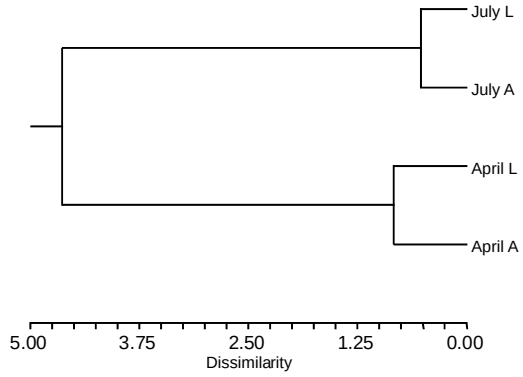
Microbial diversity indicators

There was less mass but greater diversity (H index) of the bacterial rRNA bands and greater mass of the fungal rRNA bands in the wet season soils than the dry season soils, and greater mass and diversity of the fungal rRNA bands in the L than the A soils (Table 4). Both the A and L soils had unique rDNA bacterial and fungal banding patterns, suggesting differences in microbial community structure. However, the data also show that there is much more similarity in microbial community structure when examined by season rather than soil type, showing clear shifts in microbial populations with soil moisture increases (Table 5). As well, the cluster analyses of both the bacterial and fungal diversity suggested that the diversity patterns were more similar by seasons than by soil type (Fig. 2).

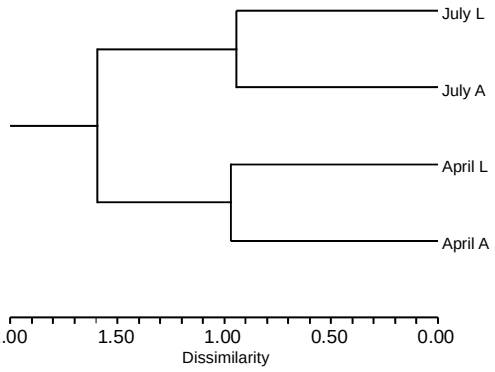
Discussion

This study showed that the metrics used can detect changes in both the nutrient and microbial community characteristics within the old growth forest soils studied. The data also provides preliminary support of the hypotheses tested in that the microbial community structure does differ bet-

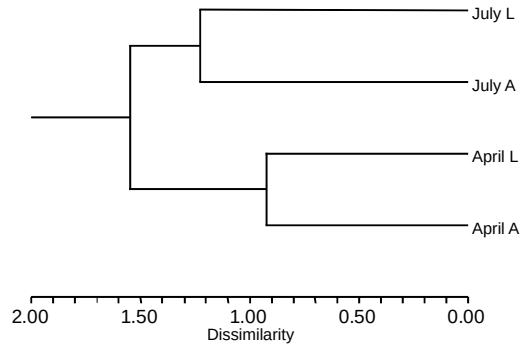
Total inorganic nitrogen (NH4-N + NO3-N) and phosphorous (P) levels, microbial biomass (mg Cmic/g), and microbial biomass efficiency (%Cmic/TOC).



Abundance of the nifH, laccase, fungal rRNA, and bacterial rRNA genes.



RFLP-based diversity analysis of bacterial rRNA following PCR amplification of soil DNA (using the 27f/1492r universal bacterial rRNA primer set) and digestion with the HhaI restriction enzyme.



RFLP-based diversity analysis of fungal rRNA following PCR amplification of soil DNA (using the EF4/Fung5 universal fungal rRNA primer set) and digestion with the HhaI and HindIII restriction enzymes.

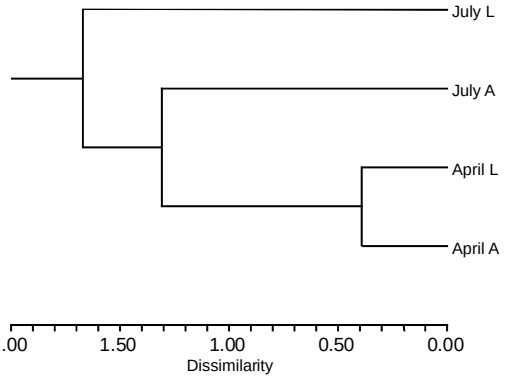


Fig. 2. Dendrograms of soil nutrient and microbial communities from A and L soil types within the Carbono Project forests in La Selva, Costa Rica, collected in April and July, 2008.

ween the younger (A) and older (L) oxisol soils; the microbial composition appears to change between the wet and dry season; and less soil microbial biomass develops due to reduced microbial activity occurring during the dry season.

Seasonal and soil type differences were found in the microbial community structure and biomass between two dominant forest soil types at La Selva, and appeared to be driven by differences occurring in the inorganic N, P and water content of the soils. Changes in soil N and P levels have been previously associated with increases in microbial activity and community structure, and soil biomass (Allison *et al* 2007; Cleveland *et al.* 2004; Cleveland *et al.* 2002; Cruz *et al.* 2009; Eaton 2001). This appears to be the case in the La Selva soils whether comparing the A to L soil types or all soils by season. This is the first study conducted in these old-growth tropical rain forests to compare aspects of the microbial community structure and biomass associated with levels of soil moisture, N, and P between two major soil types and between dry and wet seasons.

Differences in the N, P and water content occu-

Table 4. A comparison of the percent differences (PD), randomization t-test *p* values, and effect size *d* values for the cumulative total mass (as ng) of the DNA bands and the Shannon Diversity index (H) from RFLP analysis of PCR products after amplification of the DNA from A (younger oxisols) and L (older oxisols) soil types and by dry and wet season soil samples (ns = not significantly different).

	Bacterial rRNA Mass	Bacterial rRNA H	Fungal rRNA Mass	Fungal rRNA H
Type A Soil	526 (128)	2.77 (.25)	136 (39)	1.26 (.03)
Type L Soil	506 (153)	2.64 (.21)	179 (67)	1.34 (.06)
L to A Soil Type				
PD	-3.80	-4.70	24	6.00
P values	ns	ns	0.113	0.001
d values	ns	ns	0.86	2.04
Dry Season Soil	578 (168)	2.53 (.10)	114 (11)	1.29 (.04)
Wet Season Soil	457 (54)	2.90 (.18)	196 (52)	1.31 (.07)
Wet to Dry Season				
PD	-20.90	12.80	42	1.50
P values	0.057	0.0001	0.0003	ns
d values	1.03	2.68	2.31	ns

Table 5. A comparison of the number of unique rDNA bands in the different soil types and in the soils by season.

	Unique Bacterial RFLP Bands	Unique Fungal RFLP Bands
Dry Season Soil	12	0
Wet Season Soil	12	3
A Soil Type	25	2
L Soil Type	21	5

rring between the soil types and across the wet and dry season could be drivers for development of a greater fungal contribution to the microbiota, more efficient use of C, and an increase in the soil microbial biomass C. The L soils had lower levels of P and nifH gene contribution, with greater levels of inorganic N, biomass, RPC of fungal rRNA gene, and diversity of fungal rDNA RFLP bands than the A soils. This suggests that the L soils are more fungal-dominated, with greater efficiency of organic matter utilization and less need for nifH gene activity, while the A soils are more bacterial-dominated with greater contribution by N-fixing bacteria. The RFLP diversity assessment also showed that the A soils had less fungal mass and diversity of the rDNA bands.

When the data were examined by seasons, a decrease was observed in all the wet season soil sample's of inorganic N, P, and bacterial contribution to the microbiota, and an increase in microbial biomass, efficiency of utilization of C, and fungal contribution to the microbiota. In fact, the soil moisture was positively correlated with indicators of fungal-dominated soil (microbial biomass indicators, laccase gene, the ratio of fungal to bacterial rRNA), and negatively correlated with indicators of bacterial-dominated soils (bacterial rRNA RPC, nif H gene RPC and the levels of N and P nutrients). These results suggest that in the wet season, all soil types become more fungal-dominated.

The data showed there was a wet season increase in the mass and uniqueness of fungal rDNA bands, a decrease in the mass but an increase in the diversity of bacterial rDNA bands, an increase in nifH gene contribution, a decrease in inorganic N, a decrease in bacterial rRNA and an increase in fungal rRNA, and an increase in microbial biomass and efficiency of C utilization. These, collectively, suggest that selection for N-fixing bacteria is occurring along with the selection for an increase in fungi in the wet season, and also a shift towards more fungal-dominant soil community.

The diversity data also suggests that there are clear differences in the microbial community structure by soil type, but these communities become much more similar when viewed seasonally. It would be of value to determine a more detailed assessment of the microbial community structure

in these soil types, and how they change with respect to differences found in P and N-cycle components within the A and L soil types. Nevertheless, it is clear that soil moisture may be more profound than other inherent components in determining the ecological characteristics of these soils, resulting in significant shifts in community structure. Future studies should focus on the changes that occur within the major groups of microbes, concomitant with nutrient changes, during the shift from dry to wet season.

An inverse relationship between inorganic N and nifH gene abundance and activity has been shown to be due to negative feedback inhibition of N-fixation that is activated when higher concentrations of inorganic N (Daimon & Yoshioka 2001; King & Purcell 2005; Schulze 2004), or lower concentrations of P (Almeida *et al.* 2000; Eisele *et al.* 1989; Reed *et al.* 2007; Schulze 2004) are present in soil. In the current study, higher inorganic N and lower P and nifH gene levels were found in the L than A soils, suggesting that more nifH gene inhibition is occurring in the L soils.

A variety of mechanisms can cause changes in levels of inorganic N in forest soils. Some of these are consistent with the patterns observed in the current study. Coarse woody debris and litter that accumulates on the forest floors during the dry season results in an increase in inorganic N and organic matter (e.g. Condit *et al.* 1995; Engelbrecht & Kursar 2003; Mulkey & Wright 1996; Veenendaal & Swaine 1998; Wood *et al.* 2005). Reduced levels of precipitation are associated with decreases in N cycle activities, microbial activity and biomass development, and increases in concentrations of pools of inorganic N (Campo *et al.* 2001; Eaton 2001; Ewing *et al.* 2007; Luizao *et al.* 1992; McGrath *et al.* 2000). Such increases in inorganic N suppress fungal biomass development in soils (Bittman *et al.* 2005; de Vries *et al.* 2007) and the decomposition of lignin (Hobbie 2008; Knorr *et al.* 2005; Sinsabaugh *et al.* 2004; Waldrop & Zak 2006). Wood *et al.* (2005) demonstrated a decrease in leaf litter N occurred in these same A and L soils in the wet season (during July), which would have resulted in less N moving into the soil in the wet season. Consistent with this, the current study showed that inorganic N levels and RPC of bacterial rRNA were lower and microbial biomass, efficiency of utilization of SOC, RPC of fungal rRNA, and diversity and richness of fungal rRNA were greater in the wet season samples.

Some suggest that higher levels of P are associated with seasonal increases in microbial biomass and activity (Cleveland *et al.* 2002). However, McGroddy *et al.* (2004) showed that there were no differences in the rate of decomposition in tropical soils across 3-fold concentration treatments with P. Previous work in the same A and L soils indicated that greater soil respiration rates occurred during the wet season (Schwendenmann & Veldkamp 2006), presumably when the P levels would tend to be lower. The current study suggests that during the wet season there is less P, and greater biomass, contribution of fungal and laccase genes, and richness and diversity of fungal rRNA in the soils. Thus, it is clear that more work is needed concerning the role of P, soil moisture, and soil microbial activity and biomass development in these soils to confirm the patterns that may be occurring.

An increasing microbial biomass and Cmic: SOC ratio suggests more organic C is being made available to the microbial community (Anderson 2003; Moscatelli *et al.* 2005). This is often associated with an increasing fungal contribution and a relatively static bacterial contribution to the soil microbiota associated with an increase in the fungal to bacterial ratio (e.g., Griffith & Bardgett 2000; Van der Wal *et al.* 2006). In the current study, there was no difference in the bacterial contribution to the soil microbiota between the plot types, but the fungal contribution, the ratio of fungi to bacteria, and microbial biomass were much greater in the L than the A soils. In the wet season samples, there was a significant increase in fungal contribution, a decrease in bacterial contribution, and an increase in microbial biomass compared to the dry season samples. These characteristics suggest that there is a shift to a more efficient use of organic C and biomass buildup associated with more fungal dominance in the L compared to A soils, and in all soils in the wet season.

Taken collectively, the data from this study suggest key seasonal changes may be occurring in microbial community structure, diversity, and activity within these old growth forest soils that are linked to changes in the N, P, and water content. Specifically, when the nutrients from the forest floor debris are made more available with an increase in soil moisture, there is a decrease in the N and P levels, an increase in microbial biomass and efficiency of SOC utilization, along with a shift to a more fungal-dominated microbiota and an increase in fungal diversity, along with a decrease in overall bacterial contribution, but selection for N-fixing bacteria. Others have made similar observations concerning nutrient concentration

shifts (Allison *et al* 2007; Cruz *et al.* 2008). These could be the result of increased leaching or run-off due to more surface water, or that more nutrients are being incorporated into the cells associated with the increasing and more fungal-dominated biomass. These patterns could have important implications for P, N and C cycle dynamics, SOC development, lignin degradation, and C biomass development in these soils. To determine how/if seasonal environmental variations affect these parameters, and, perhaps more long-term, how changing climatic conditions affect biomass development in the soil microbial communities, it will be necessary to study these soils over the wet and dry seasons and for several years.

Conclusions

This preliminary study illustrates the tremendous need for more work in these soils to clarify questions concerning changes in the microbial community structure and function, particularly related to issues of microbial community composition and C biomass development, N and P concentrations and how all these are influenced by soil moisture. A critical result from this work, however, is the demonstration that the soil community shifts away from fungal and towards bacterial contribution, with less belowground incorporation of C into biomass in the dry season. It is not known yet if the annual amount of belowground C biomass development is decreasing in concert with other climate-related changes observed by others in tropical forests (Clark 2007; Clark *et al*. 2003; Feeley *et al*. 2007; Lewis 2006; Wright 2005). However, we believe this to be a plausible hypothesis that warrants further and sustained attention.

To confirm the trends we have observed, our future project work will include assessment of the levels of microbial biomass, N, P, and efficiency of C utilization, and abundance and diversity of specific groups of microbes and critical genes associated with both C and N cycle activities over space and time. If the results of our study are indeed part of a larger trend, there are significant implications for future decisions on climate change policy, ecosystem dynamics studies, and tropical forest modeling.

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