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Implications of land management on soil microbial communities and nutrient cycle dynamics in the lowland tropical forest of northern Costa Rica

K. HAFICH[1], E. J. PERKINS[2], J. B. HAUGE[3], D. BARRY[3] & W. D. EATON[4*]

[1]*Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, NM 87131-001, USA*

[2]*Biology Department, University of Puget Sound, Tacoma, WA 98416-1088, USA*

[3]*Peninsula College, Center of Excellence in Environmental Science and Natural Resources, and Western Washington University Huxley College of the Environment at Peninsula College, Port Angeles, WA 98362 USA*

[4]*School of Environmental and Life Sciences, Kean University, Union, New Jersey 07083, USA*

Abstract: The long-term effects of reforestation versus maintained grassland on microbial community structure and nutrient cycling provide a valuable perspective on ecosystem health and carbon sequestration potential of tropical soils in the heavily deforested Northern Zone of Costa Rica. The soil from the secondary forests in this area had greater levels of phosphate, inorganic nitrogen, organic carbon, respiratory activity, abundance and diversity of Basidomycete rDNA, abundance of fungal rDNA, and lower abundance but greater diversity of *Rhizobium* rDNA, and less abundance of nifH gene DNA than soils from adjacent grasslands of the same age. Critical correlations were observed between the abundance of Basidiomycete rDNA and laccase gene with the levels of phosphate, microbial biomass, organic carbon use efficiency, and percent water saturation. These data suggest a trend towards the secondary forest soils becoming more fungal-dominant, with greater microbial activity, greater nitrogen mineralization activity and more efficient use of carbon. This project provides some of the first evidence that the management strategy of regeneration of secondary forests results in more complex soil ecosystems, with greater potential for carbon sequestration than the maintained grasslands.

Resumen: Los efectos a largo plazo de la reforestación contra los del mantenimiento de pastizales sobre la estructura de la comunidad microbiana y el reciclaje de los nutrientes ofrecen una perspectiva valiosa sobre la salud del ecosistema y el potencial para secuestrar carbono de los suelos tropicales en la zona norte de Costa Rica, fuertemente deforestada. El suelo de los bosques secundarios en esta área tuvo niveles más altos de fosfato, nitrógeno inorgánico, carbono orgánico, actividad respiratoria, abundancia y diversidad de ADNr de basidiomicetos, abundancia de ADNr fúngico, y una menor abundancia pero una mayor diversidad de ADNr de *Rhizobium*, así como una abundancia menor de ADN del gen nifH que los suelos de pastizales contiguos de la misma edad. Se observaron correlaciones críticas entre la abundancia de ADNr de basidiomicetos y del gen de la lacasa con los niveles de fosfato, la biomasa microbiana, la eficiencia del uso del carbono orgánico y el porcentaje de saturación de agua. Estos datos sugieren que los bosques secundarios tienden a estar más dominados por hongos y a tener una mayor actividad microbiana, una mayor actividad de mineralización del nitrógeno y un uso más eficiente del carbono. Este proyecto proporciona algunas de las primeras

* *Corresponding Author*; e-mail: weaton@kean.edu

evidencias de que la estrategia de manejo de la regeneración de los bosques secundarios resulta en ecosistemas edáficos más complejos, con un mayor potencial para el secuestro de carbono, que el mantenimiento de los pastizales.

Resumo: Os efeitos de longo prazo da reflorestação, versus manutenção da pastagem na estrutura da comunidade microbiana e no ciclo de nutrientes, proporcionam uma perspectiva valiosa sobre a sanidade do ecossistema e no potencial de sequestração de carbono de solos tropicais na região fortemente desflorestada na zona norte da Costa Rica. O solo das florestas secundarias nesta área apresentava altos teores de fosfato, azoto inorgânico, carbono orgânico, atividade respiratória, abundância e diversidade de rDNA de Basidiomicetas, abundância de rDNA fúngico e pouca abundância mas grande diversidade de rDNA de *Rhizobium* e menos abundância do gene nifH do DNA do que nos solos das pastagens adjacentes da mesma idade. Foram observadas correlações críticas entre a abundância do rDNA de Basidiomiceta e do gene da lacase com os níveis de fosfato, da biomassa microbiana, a eficiência de uso do carbono orgânico, e a percentagem da saturação de água. Estes dados sugerem a tendência para que os solos da floresta secundária se apresentarem com uma maior dominância da componente fúngica, com maior atividade microbiana, maior atividade de mineralização do azoto e um uso mais eficiente do carbono. Este projeto providenciou alguma da primeira evidência de que a estratégia de gestão da regeneração resulta em ecossistemas de solo mais complexos, com maior potencial para a sequestração de carbono do que as pastagens mantidas.

Key words: Carbon sequestration, Costa Rica, Maquenque National Wildlife Refuge, nitrogen, nutrient cycling, soil microbial communities, tropical reforestation.

Introduction

The effect of tropical rainforest deforestation has reached across the globe, at scales ranging from atmospheric to microbial communities and geochemical properties of tropical soils. Globally, deforestation has been documented for several decades as contributing to an atmospheric rise in greenhouse gas levels (e.g. Fearnside 2000), changes in release of trace gases (e.g. Keller *et al.* 1993) and in hydrological processes (e.g. Meher-Homji 1991), and significant losses of biodiversity at many trophic levels (e.g. Ehrlich & Wilson 1991). After forests are cleared, whether land is managed grassland, allowed to regenerate as secondary forest, or used for other purposes, has substantial impact on vegetation, nutrient availability, and microbial communities (Cleveland *et al.* 2003; Decaëns *et al.* 2006; Nüsslein & Tiedje 1999; Reiners *et al.* 1994).

Costa Rica experienced significant deforestation in the twentieth century, as well as reforestation in the last 40 - 50 years (Chassot *et al.* 2001, 2005; Monge *et al.* 2002, 2003). Between 1941 - 1983, Sader & Joyce (1988) estimate that 83 % of forested land in Costa Rica was cleared, with disproportionally more habitat loss in the tropical lowlands. From the mid-1980s to the mid 1990s, reforestation in the Northern lowlands of Costa Rica balanced out deforestation, with 7.3 % of the landscape being reforested and 7.9 % of land being converted from forest to pastures (Powers 2004).

Wardle (2006) recognized the need to identify the linkages between above and below-ground diversity, the roles of nutrient resource availability, and terrestrial habitat properties, yet few studies have connected these together in habitat assessments, especially within tropical ecosystems. Chazdon *et al.* (2007) discussed the rates of changes in vegetation occurring in some of the lowland forests of Costa Rica following the original disturbance leading to the development of older, well-functioning secondary forests. However, the composition and function of the microbial community in such secondary forests in this part of Costa Rica has not been addressed to date. Many studies have shown that the assessment of microbial community and nutrient cycle dynamics can be used as indicators of a healthy soil ecosystem with potential value for assessment of ecosystem management, restoration, and conservation strategies employed in the secondary forests of the tropics (e.g.

Buckley & Schmidt 2003; Hartmann & Widmer 2006; Ibekwe *et al.* 2007; Wardle *et al.* 2004). As reforestation becomes more common in the tropics, the examination of the microbial community and associated nutrient cycle components could be used to monitor soil ecosystem development to assess the efficacy of this and other land management practices. Thus, the objective of this study was to compare the long-term effects of forest clearing followed by grassland maintenance versus secondary forest regeneration on components of the soil ecosystems in the Northern Zone of Costa Rica.

Materials and methods

Study sites and sample collection

The study sites used were within the Maquenque National Wildlife Refuge (MNWLR) in Costa Rica, which is located in Northeast region of Costa Rica, about 15 km south of the Nicaraguan border (10° 27'05.7"N, 84° 16' 24.32"W). The sites had been primary forest that was cleared in the early 1980's. Part of the land was allowed to regenerate into a mixed species secondary forest and part was established as pasture for cattle. The cattle were removed from the pasture beginning in 1991, and the pasture was then maintained as grassland.

Four plots of 1000 m^2 were established in the secondary forest and the grasslands, and divided into ten 10 x 10 m subplots. Within each subplot, eight randomly located 2 cm diameter x 15 cm deep cores were collected and then composited by subplot on two consecutive days in July, 2009. This resulted in four discrete soil samples per habitat type, made up of 80 soil cores (10 subplots x 8 cores) that were then analyzed. Soil was collected using sterile technique to avoid cross contamination between plots. Separate samples were collected at each subplot for bulk density determination, and the percent saturation and pH were also determined at 10 randomly located sites within each plot using a Kelway HB-2 Soil and pH meter (Wyckoff, NJ, USA). Samples were mixed and sieved at 10 mm to remove rocks, insects and plant matter. All data were adjusted for soil moisture levels, dry weight, and bulk density to standardize results to a common soil volume (Cleveland *et al.* 2003; Powers 2004).

Nutrient analysis

Nutrient analyses were conducted within two days of soil collection. Nitrate (as NO_3-N) and ammonium (as NH_4-N) content were measured using 50 mL of 2M KCl extraction of 10 g of soil (Alef & Nannapieri 1995) followed by ammonium salicylate and cadmium reduction spectrophotometric methods using the HACH DR 2700 system (Hach Company, Loveland, Colorado, 80539-0389; HACH methods 8155 and 8192 respectively). The total mineral nitrogen (TMN) levels were estimated as the sum of the NO_3-N and NH_4-N values. Phosphate (PO_4) was measured after Bray 1 extraction from 2 g of soil using the molybdenate reduction method (HACH method # 8048) and the HACH DR 2700 system.

The microbial biomass C (C_{mic}) was determined for each sample by the fumigation-extraction method (Jenkinson 1988) as the difference between the 0.5 M K_2SO_4-extracted soil dissolved organic carbon (DOC) levels in ethanol-free chloroform-fumigated and unfumigated 10 g soil subsamples. The DOC 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 rate of respiration was determined as CO_2 released using a Qubit SR1LP Respiration system (Kingston, ON, Canada). The microbial metabolic quotients (qCO_2, as a ratio of CO_2 from respiration/C_{mic}) and the ratio of C_{mic}/SOC were determined to estimate the efficiency of utilization of organic C (Anderson 2003).

DNA analysis

DNA was extracted from soil samples within five days of collection using the Power Soil DNA Isolation kit (MO BIO, Carlsbad, CA). Three replicates were used for each of the four plots per habitat type, using 0.3 g of soil for each extraction, after which the replicates were pooled for PCR analysis. DNA was stored at 4 °C until use. End-point PCR (using an Applied Biosystems, Foster City, CA 9700 Thermal Cycler) was used to identify the presence of target genes in each sample. The primers referenced in Table 1 and AmpliTaq Gold (Applied Biosystems, Foster City, CA) complete Master Mix were used for the PCR assays, along with the suppliers recommend PCR temperature schedule, except that for laccase, nifH, Basidiomycete ITS, and 16S *Rhizobium* PCR assays, the annealing temperature was 55 °C; and the 18s fungal rRNA annealing temperature was 48 °C. The relative abundance (RA) of each target gene was estimated using a quantitative PCR assay using the above primers and conditions and a MJ Research Opticon One thermal cycler and

Table 1. PCR primers used to identify the presence and diversity of target genes in soils from secondary forests and adjacent grasslands in the Northern Zone of Costa Rica.

Gene	Primer	Sequence	Reference
nifH gene	nifHf	TAC GG**N** AAR GG**S** GG**N** ATC GGC AA	Grange *et al.* 2007
	nifHI	AGC ATG TC**Y** TC**S** AG**Y** TC**N** TCC A	
Rhizobium 16S	63f	CTC GCT GCC CAC TGT CAC	Singh *et al.* 2006
	Rhiz12 44r	AGG CCT AAC ACA TGC AAG TC	
Fungal 18S	EF4	GGA AGG G**R**T GTA TTT ATT AG	Smit *et al.* 1999
	EF3	TCC TCT AAA TGA CCA ACT TTG	
Basidiomycete ITS	ITS1F	CTTGGTCATTTA GAGGAAGTAA	Dickie & FitzJohn 2007
	ITS4	TCCTCCGCTTAT TGATATGC	
Laccase-gene	Lac2f	CGC ATC ATC TTT TGT GCT CC	Litvintseva & Henson 2002
	Lac2r	AGC GCA ACT ACG ACG AGG A	

*Boldface letters signify degenerate positions. N, A+C+G+T; R, A+G; S, G+C; Y, C+T.

Table 2. Restriction endonucleases used in Restriction Fragment Length Polymorphism (RFLP) assays to estimate the diversity of target genes in soils from secondary forests and adjacent grasslands in the Northern Zone of Costa Rica.

Gene	Enzymes
nifH gene	HaeIII/MspI/MboI/HhaI
Rhizobium 16S r RNA	HhaI/MspI
Fungal 18S rRNA	HinfI/TaqI
Laccase gene	HhaI/MspI/TaqI
Basidiomycete ITS	HaeIII/HinfI/AluI/Taq

Thermo Scientific SYBR Green Master Mix in which the abundance of the PCR product DNA from the soil samples was compared to known concentrations of purified target gene DNA (6.5 to 28.4 ng μL^{-1} of cloned target gene DNA with sequences confirmed in GenBank), from which the target DNA concentrations in each sample were determined, and the RA calculated. The percent of the total RA that each target gene represented within a sample was determined and reported. A restriction fragment length polymorphism (RFLP) assay was performed on the PCR amplified DNA from each sample to provide a preliminary estimate of the target gene diversity. The restriction endonucleases were from Fermentas, Inc. (Burlington, ON, Canada) and are listed in Table 2. The digestions were performed using the methods suggested by the vendor and a known concentration of PCR product (approximately 0.2 µg). The resulting size and quantity (per µg of starting PCR product) of each DNA band (in 2 % agarose electrophoresis) were determined for each DNA sample using GeneTools software (Syngene, Frederick, MD), and converted into Shannon-Weiner (Shannon & Weaver 1963) richness (S), diversity (H′), and evenness indices by soil type.

Statistical analysis

A weight of evidence statistical approach was used to compare differences in the mean values of all metrics determined from the four pooled soil of each habitat type. The percent differences (PD), t-test *P* values, and the Cohen's *d* effect size values were used to suggest biologically meaningful differences between means, consistent with the recommendations for analysis of small sample sizes by Di Stefano *et al.* (2005). We used approximate t-test P values ≤ 0.25, PD ≥ 10, and Hedge's d values ≥ 0.7 (> 0.7 is considered a large effect size difference) to define biologically meaningful differences in the means for this project.

Following analysis of the data for differences between habitats, the data from all habitats was aggregated (n = 8) and analyzed by Pearson's Correlation methods to determine the strength of possible relationships that will serve as soil ecosystem condition indicator targets in future studies. Any two factors resulting from this analysis with r values > 0.443 or ≤ -0.443, with P values < 0.2 were considered critical correlations based on standard tables of critical values of r. Since increases in the abundance of Basidiomycetes have been identified as an indicator of soil complexity (Anderson 2003; Baldrian 2006; Sinsabaugh 2010), we calculated the correlation coefficients between the RA of the laccase gene (indicating potential lignin degradation by Basidomycetes) and the RA

Table 3. A comparison of the physical, nutrient and microbial activity parameters in soil collected from grasslands and adjacent secondary forests in Costa Rica.

	Secondary Forest	Grassland	PD Sec. to Grass.	*P* value	Hedge's *d*
Soil Physical Characteristics					
pH	6.4 ± .2	6.3 ± 0.3	1.6	0.6	0.4
% Saturation	49.7 ± 3.9	52.3 ± 3.7	-2.6	0.51	0.53
Bulk Density (g cm^{-3})	0.7 ± 0.1	0.76 ± 0.05	-7.9	0.32	0.76
Soil Nutrient Characteristics					
Mineral N (μg cm^{-3})	6.1 ± 0.9	4.4 ± 0.4	37.6	0.02	2.32
NO_3-N (μg cm^{-3})	1.4 ± 0.3	0.5 ± 0.2	178.8	0.004	3.25
NH_4-N (μg cm^{-3})	4.6 ± 1.0	3.9 ± 0.3	18.7	0.23	0.95
% N as Nitrate	24.3 ± 7.4	11.4 ± 4.4	113.2	0.02	2.12
Phosphate (μg cm^{-3})	8.8 ± 1.4	7.8 ± 0.5	13	0.22	0.98
Organic C (μg cm^{-3})	593 ± 35	532 ± 47	11.5	0.08	1.47
Soil Carbon Use Characteristics					
Biomass C (μg cm^{-3})	301 ± 34	219 ± 194	37.4	0.44	0.5
Respiration (mg g^{-1} h^{-1})	3.7 ± 0.4	2.8 ± 1.4	32.1	0.25	0.87
qCO_2 (Resp/Cmic)	0.08 ± 0.01	0.14 ± 0.16	-42.9	0.48	0.53
Cmic/SOC	0.51 ± 0.05	0.43 ± 0.40	18.6	0.71	0.28

PD = percent difference in mean values; *P* = t-test *P* value; Hedge's *d* effect size value.

Table 4. A comparison of the mean percent relative abundance (% RA) values (± standard deviation) of *Rhizobium* 16S rRNA, nifH gene, laccase gene, and fungal 18S rRNA in secondary forest and *Pentaclethra*-dominant forest soils within the Maquenque National Wildlife Refuge in the Northern Zone of Costa Rica.

	% RA Rhizobium	% RA nifH	% RA Laccase	% RA Fungus	% RA Basidiomycetes
Grassland Soil (n = 4)	27.2 ± 9.4	25.1 ± 12.5	10.9 ± 0.04	6.5 ± 4.3	45.0 ± 12.7
Secondary Forest Soil (n = 4)	4.7 ± 1.6	12.6 ± 14.9	9.3 ± 5.4	24.9 ± 19.6	55.1 ± 9.2
PD	-82.7	-49.8	-7.9	283.1	21.9
P value	0.01	0.25	0.7	0.12	0.25
Cohen's *d* value	3.06	0.91	0.3	1.3	0.89

PD = percent difference in mean values; *P* = t-test *P* value; Hedge's *d* effect size value.

of Basidiomycete rDNA with C_{mic}, C_{mic}/DOC, qCO_2 as indicators of efficiency of C use (Anderson 2003); and PO_4, as some have suggested that the level of microbial activity increases with increases in this nutrient (Allison *et al*. 2007; Cleveland *et al*. 2004; Cleveland & Townsend 2006; Cruz *et al*. 2009; Eaton *et al*. 2011; Townsend *et al*. 2002).

Results

There were no meaningful differences between the pH, percent water saturation, and bulk density between the grassland and secondary forest sites. The NO_3-N, NH_4-N, and total mineral N (TMN) levels were greater in the secondary forest than grassland soils, and the percent of TMN as NO_3 was much greater in the secondary soils (Table 3) . The levels of inorganic P and the respiration rate were also greater in the secondary forest soils (Table 3).

The abundance of the *Rhizobium* rDNA and nifH gene DNA were greater in the grassland soils than in the secondary forest soils, but the diversity, richness, and evenness of distribution of the

Table 5. RFLP-based Shannon-Weaver diversity indices for *Rhizobium* 16S rDNA, fungal 18S rDNA, and Basidiomycete ITS DNA from grassland and secondary forest soils within the Maquenque National Wildlife Refuge in the Northern Zone of Costa Rica. The Shannon-Weaver diversity index values are for richness (*S*), diversity (H′), and evenness of distribution (*E*).

Rhizobium 16s rRNA	Grassland	Secondary Forest	PD	*P* value	*d* value
H′ index	0.66 ± 0.36	1.55 ± 0.35	40.3	0.02	2.06
S index	2.75 ± 0.96	6.0 ± 2.58	37.1	0.06	1.45
E index	0.67 ± 0.27	0.89 ± 0.05	14.1	0.16	0.98
Fungal 18s rRNA					
H′ index	1.98 ± 0.27	1.72 ± 0.05	-7.0	0.11	1.16
S index	9.33 ± 1.53	7.67 ± 1.15	-9.8	0.13	1.07
E index	0.89 ± 0.06	0.85 ± 0.03	-2.3	0.28	0.73
Basidiomycete ITS					
H′ index	0.7 ± 0.7	1.55 ± 0.35	37.8	0.06	1.34
S index	2.85 ± 1.71	5.50 ± 1.73	92.9	0.07	1.54
E index	0.52 ± 0.35	0.94 ± 0.01	28.8	0.06	1.41

PD = percent difference in mean values; *P* = t-test *P* value; Hedge's *d* effect size value.

Table 6. Critical correlation results (defined as having r values ≥ 0.443 or ≤ -0.443, with P values ≤ 0.2) from comparisons between certain parameters measured in the soil in secondary forest and grassland soils within the Maquenque National Wildlife Refuge in the Northern Zone of Costa Rica. Pearson's Correlations were made between phosphate (PO_4), microbial biomass (C_{mic}), carbon use efficiency metrics (C_{mic}/DOC and qCO_2), and the relative abundances (RA) of laccase gene and Basidiomycete ITS DNA. Only the r values and associated P values (in parentheses) of the critical correlations are presented (NC = not critical).

	PO_4	C_{mic}	C_{mic}/DOC	qCO_2
RA Basidiomycetes	0.580(0.132)	0.715(0.046)	0.703(0.052)	-0.526(0.181)
RA Laccase	0.469(0.093)	0.453(0.196)	NC	-0.687(0.060)

Rhizobium rDNA were greater in the secondary forest soils (Table 4). The abundance of both the fungal and Basidiomycete rDNA were greater and the diversity, richness, and evenness of the Basidiomycete rDNA was also greater in the secondary forest soils (Table 4). However, the diversity, richness and evenness of the fungal rDNA was somewhat greater in the grassland than secondary soils, and there were no differences in the laccase gene abundance and diversity between habitats (Table 5).

Critical positive correlations were found between the RA of the Basidiomycete rDNA and P and C biomass levels, and the ratio of C biomass to DOC, and a critical negative correlation was found between the Basidiomycete rDNA with the qCO_2 (Table 6). Similar critical correlations were found between the RA of the laccase gene DNA and these same parameters, except there was no critical correlation with the ratio of C biomass to DOC (Table 6).

Discussion

The evaluation of the soil ecosystem metrics measured in this study have potential value for assessment of ecosystem management, restoration, and conservation strategies employed in the secondary forests of the tropics, and for prediction of changes in the amounts of C and N sequestered into the biota (Buckley & Schmidt 2003; Ibekwe *et al*. 2007; Nüsslein & Tiedje 1999; Oelbermann *et al*. 2004; Wardle *et al*. 2004). The greater respiratory activity, fungal rDNA abundance, and Basidiomycete DNA abundance and diversity, phosphate, inorganic N, and organic C, suggest that the secondary forest soil microbial community was more complex, fungal dominant, and active than in the grasslands, with more efficient use of C and greater potential for C sequestration, which is consistent with a more well-established and complex soil ecosystem (Anderson 2003). However, it is also possible that there may be differences in these

relationships that occur between wet and dry season. To clarify this, we have begun a wet season versus dry season study of the same plots and parameters in the hope of determining the effect that both land management and season has on these soil ecosystems.

The greater amount of P in the secondary soils being associated with greater microbial activity and other indicators of a more complex soil ecosystem is consistent with the work of others in similar forests (Allison *et al.* 2007; Anderson 2003; Cleveland *et al.* 2004; Cleveland & Townsend 2006; Cruz *et al.* 2009; Eaton *et al.* 2011; Townsend *et al.* 2002). After conversion of tropical rain-forest to pasture, inorganic P declines, and organic P levels either remain constant or increase (Townsend *et al.* 2002), while microbial efficiency of organic C use and P mineralization decreases in the long term (Cleveland *et al.* 2002; Townsend *et al.* 2002). Available P is a critical limitation of microbial C use (Cleveland *et al.* 2002), and consequently higher levels of P often result in increased microbial biomass (Cleveland *et al.* 2004) and increased microbial respiration (Cleveland & Townsend 2006).

The greater amount of inorganic N and percent of inorganic N as nitrate, along with the lower abundance of the *Rhizobium* rDNA and nifH genes in the secondary forest soils may be the result of the recognized feedback inhibition effect that elevated levels of inorganic N have on decreasing the rates of N-fixation (Daimon & Yoshioka 2001; King & Purcell 2005; Pons *et al.* 2007; Reed *et al.* 2007; Schulze 2004). These data also suggest the possibility that there may be greater efficiency of N-fixation, and perhaps rates of ammonium oxidation occurring in these secondary forest soils (Booth *et al.* 2005; Sahrawat 2008; Webster *et al.* 2002). As well, other have shown lower rates of nitrification occurred in pastures as compared to forests in similar tropical areas (Carney *et al.* 2004; Cleveland *et al.* 2003; Reiners *et al.* 1994), which can be due to a greater rate of assimilation of N into the herbaceous biomass typically found in grasslands, where N turnover is fastest (Booth *et al.* 2005).

It is interesting that the greater levels of inorganic N were also associated with greater abundance of fungal and Basidiomycete rDNA. Although it has been well-established that higher levels of inorganic N can inhibit soil fungi in general, and the Basidiomycetes in particular (Bittman *et al.* 2005; de Vries *et al.* 2007; Dighton *et al.* 2004; Dijkstra *et al.* 2004; Hobbie 2008; Knorr *et al.* 2005; Waldrop & Zak 2006), it is also recently documented that nitrification can be carried out by a number of species of fungi, especially in acidic forest soils (Dighton *et al.* 2004; Hayatsu *et al.* 2008), and that shifts can occur in forest soils from "nitrophobic" fungal species to more "nitrophilic" species resulting in a fungal population less susceptible to inhibition by inorganic N (Dighton *et al.* 2004). Both of these fungal characteristics could account for the greater fungal abundance levels in the presence of soils with high levels of inorganic N.

Microbial community diversity and structure is inextricably linked with nutrient parameters of tropical soils and above-ground vegetation affected by land use and management (Anderson 2003; Cleveland *et al.* 2003; Reiners *et al.* 1994). Conversion of secondary forest to grassland soils has been associated with decreases in the size and complexity of the microbial community (e.g. Bornemann & Triplett 1997; Cleveland *et al.* 2003). In the current study, there was greater abundance and dominance (i.e. lower E values) and lower diversity of *Rhizobium* rDNA and nifH gene DNA in the grasslands, and lower abundance of fungal and Basidiomycete rDNA in the grassland soils than the secondary forest soils. This, along with the nutrient data, support the suggestion that the secondary forests were more fungal dominant, with more organic C being produced and used more efficiently than in the grasslands.

An increased abundance of Basidiomycete fungi and laccase activity associated with lignin degradation has been associated with and enhancement of soil ecosystem complexity (Anderson 2003; Baldrian 2006; Sinsabaugh 2010). Our correlation analyses support this in that the biomass C and the indicators of C use efficiency were correlated appropriately with both the RA of Basidiomycete rDNA and lacacse DNA. There was also a critical correlation between the levels of phosphate and the RA of Basidiomycete rDNA and laccase DNA, which is consistent with earlier work conducted by Eaton *et al.* (2011), who demonstrated an increase in fungal activity in similar region tropical soils when phosphate levels were increasing. These results are encouraging for us and provide targets for extended work on developing models to predict soil quality in this region of Costa Rica.

Significant deforestation and land degradation in the tropics has resulted in increased rates of carbon dioxide and methane emissions to the atmosphere, loss of biodiversity, and a decrease in C sequestration capacity. In response to this degradation, land management strategies in the tropics

are changing from intensive use to reforestation in order to protect the large amount of C stored in these soils (Jobbágy & Jackson 2000). The development of secondary forests is becoming an important strategy in the tropical terrestrial regions for increasing the amount of soil carbon, carbon sequestration (Guo & Gifford 2002; Post & Kwon 2000; Wright 2005), and forest ecosystem services in general (see Chazdon 2008 for review). To confirm the success of such strategies requires the assessment of the impact that these restoration methods have on forest ecosystems. We suggest that a combination of nutrient chemistry, microbial biomass and activity measurements, and molecular microbiological evidence should be used to assess forest restoration success in the tropics and also for development of predictive models to allow more informed land management decisions.

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