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The Effects of the N-Fixing Tree *Pentaclethra macroloba* on the Above and Below Ground Communities Within a Primary Forest in the Northern Zone of Costa Rica

W.D. Eaton, D. Shebitz, K. Niemiera and C. Looby
Kean University, School of Environmental and Life Science,
New Jersey
USA

1. Introduction

The soil microbial community is not only critical to nutrient recycling and mineralization of organic material, but also plays a fundamental role in influencing plant community composition (Kent & Triplett 2002, Buckley & Schmidt 2003, Leininger *et al.* 2006, Wardle *et al.* 2004, Ibekwe *et al.* 2007, Fierer *et al.* 2007, Litton & Giardina 2008). Conversely, differences in vegetation community also affect the soil fungal and bacterial composition, which then drives the decomposition and nutrient cycle processes. For example, a complex relationship exists between vegetation-derived carbohydrates, lignin, soil inorganic N, increased soil complexity and microbial community development that affects the soil organic C levels and productivity (Guggenberger et al. 1994, Zech and Kögel-Knaber 1994, Brookes 1995, Anderson 2003, He et al. 2003, Moscatelli et al. 2005, Bradford et al. 2008). Furthermore, increases in soil inorganic N stimulate increased production of more labile root-derived carbohydrates by plants which are used by the bacterial community, while preliminary plant decomposition selects for fungi which degrade the lignin, cellulose, hemicelluloses, and other complex materials (Guggenberger et al. 1994, 1995; Padmanabhan et al. 2003; de Boer et al. 2005; Fierer et al. 2007; Bradford et al. 2008; Talbot et al. 2008). Thus, it is the fungi that decompose more complex organic substrates more efficiently than bacteria leaving behind more recalcitrant residues and enhancing the organic carbon matter in the soil, and are more important as decomposers in older or restored soils (Holland and Coleman, 1987, Bardgett et al. 1993, Cambardella and Elliot 1994, Guggenberger et al. 1994, 1995, 1999; Beare 1997, Bardgett and McAlister 1999; Stahl et al. 1999, Griffith and Bardgett 2000, Zeller et al. 2001; Bailey et al. 2002, Talbot et al 2008). The soil bacteria, on the other hand, are more critical in decomposition and N nutrient cycling in managed, young, or recovering ecosystems (Moore and de Ruiter 1991, Lovell et al. 1995).

Many scientists have recently recognized the value in studying the linkages between above and below-ground communities in order to better understand nutrient resource availability and the dynamic processes associated with nutrient cycling and terrestrial ecosystem

function and condition (Wardle et al 2004, Bardgett et al 2005, Wardle 2006, Fierer et al. 2010, Kardol and Wardle 2010). It is becoming clear that the simultaneous assessment of the soil biota and above ground vegetation communities, and the associated nutrient components can shed light on many questions and issues associated with terrestrial ecosystem condition, the nutrient cycle processes, drivers of forest succession, and ecosystem remediation following disturbance. The below-ground indicators of organic matter decomposition and utilization, the N cycle processes, soil carbon (C) and nitrogen-(N) biomass and rates of development, and the relative contribution of fungi and bacteria are of special interest in understanding ecosystem dynamics especially when examined in concert with above-ground vegetation metrics (Anderson 2003, Buckley & Schmidt 2003, He *et al.* 2003, Carney *et al.* 2004, Moscatelli *et al.* 2005, Pendall *et al.* 2008). However, more information is needed to connect the soil and vegetation parameters together and also to connect them with ecosystem functioning (Anderson 2003, Carney & Matson 2005, Wardle 2006, Pendall *et al.* 2008).

Most of the scientific efforts associated with these findings have occurred in either temperate forests or agricultural systems, with far too little work occurring in tropical forest ecosystems. The paucity of research in the tropics is remarkable when one considers that tropical forests contain some of the greatest biodiversity and biomass on the planet, 20% of the planet's C within the first 3 m of their soil, and are considered one of the most critical biogeographic zones for global nutrient cycling and C and N sequestration (Jobbágy and Jackson, 2000). As such, understanding how vegetation and soil fungal and bacterial communities interact to drive the decomposition and nutrient cycle processes in the tropics is especially critical now as deforestation of these unique and critical habitats has resulted in deleterious impacts at local, regional and global scales, including an atmospheric rise in greenhouse gas levels (Keller et al 1993, Cochrane and Laurance 2002, Laurance and Peres 2006), shifts in biodiversity (Ehrlich and Wilson 1991), and negative impacts on nutrient cycles (Reiners et al 1994, Nüsslein and Tiedje, 1999, Cleveland et al 2003, Decaëns et al 2006), thus, representing a great threat to the global nutrient cycles and C and N sequestration (e.g.,Laurance et al 1997, Wolters et al 2000, Campo et al 2001, Carney et al 2004, Feldpausch et al 2004, Waldrop and Firestone 2006, Clark 2007, Ewing et al 2007, Sahrawat 2008). Yet, little is known of the important factors associated with establishing, maintaining, and/or changing the level and rates of activity of the biogeochemical nutrient cycles within tropical forest communities, ecosystems, and landscapes. These factors may be biotic (e.g., adundance of nitrogen-fixing microbes in soil, fungal dominance in soil, plant species and percent cover) or abiotic (e.g., fire, moisture, temperature, soil texture). Whatever their nature, these factors must be critical in shaping and maintaining ecosystem conditions, yet there is a large gap in the knowledge about these drivers of nutrient processes within tropical ecosystems.

One such set of biotic factors presumed to be important in ecosystem development and condition in tropical forests are the symbiotic and free-living bacteria and fungi associated with the so-called "N-fixing" trees and understory vegetation. However, there is little information regarding the effect of this group of plants and the associated microbial community on above and below ground biotic community structure and function, and on nutrient cycle processes and dynamics in tropical forest ecosystems around the world. This lack of information is despite the knowledge that plants with N-fixing symbiotic root

nodules, bacteria and archaea associated with nitrification, and both saprotrophic and ecotmycorrhizal fungi represent a biotic consortium that play critical roles in forest ecosystem function, which can be affected by changes in these biotic communities.

Pentaclethra macroloba is a member of the Fabaceae, and considered a dominant N-fixing tree in Central American hardwood forests. As such, this tree is thought to be an early succesional tree that is important in N and C cycle dynamics and biomass enhancement, and ecosystem development in these forests (Hartshorn & Hammel 1994, Wang and Qui 2006, Pons *et al.* 2007). Despite the critical roles this species is thought to play, the above and below ground community structure and function associated with it is poorly characterized at best, and generally under-studied. Eaton et al (2012) provided the first preliminary look at the composition of the soil community associated with these trees in the tropical lowlands of Costa Rica. They found that *Frankia, Rhizobium,* Archaea, and Type II methanotrophs were present and likely involved in recuperating the soil N and enhancing the microbial biomass C via more efficient use of organic C. To our knowledge, there have been no studies that assess the role of *P. macroloba* in development of both the understory vegetation and the associated bacterial, fungal, and archaeal communities in these primary forests; how the structure, abundance and biomass of *P. macroloba* may drive the structure of the understory plant and microbial communities; and how these collectively may drive the C and N cycle dynamics in these soils. These concepts were the focus of our project reported here, which represents the first attempt to identify the role of *P. macroloba* on the nearby above and below ground ecosystem in tropical primary forests.

This work was conducted in two distinct habitats that are located in the San Juan-La Selva Biological Corridor, which is in the Northern Zone forests of Costa Rica: a primary forest dominated by *P. macroloba* and a primary forest with very little of these trees. To determine the role of *P. macroloba*, as the dominant N-fixing tree in these tropical forests, we compared the following components within the 2 different habitats:

- the composition of the understory vegetation (N-fixing and non-N-fixing);
- the DNA-based abundance of the fungal and bacterial DNA, the ammonium oxidizing bacteria, methyltroph, and Archaea DNA;
- the C and N cycle dynamics, rates of production of NH_4 and NO_3;
- the soil dissolved organic C and total N levels, and the C:N ratios;
- The amounts of Phosphorus (P) and the N:P ratios;
- the amount and rates of development of production of C and N biomass;
- the density and percent cover of *P. macroloba* in the two stands;
- the richness and evenness of distribution of the understory vegetation; and
- the richness and evenness of the N-fixing understory vegetation.

2. Methods

2.1 Field locations

In 2001, the Costa Rican Ministry of Environment and Energy helped establish the San Juan-La Selva Biological Corridor (SJLSBC) to help protect the Northern Zone ecosystems (http://www.lapaverde.org.cr) from further damage due to 3-4 decades of extraction-based land management practices (Monge *et al.* 2002). The core conservation unit of the SJLSBC is

the Maquenque National Wildlife Refuge (MNWLR; Figure 1), which is located in Northeast region of Costa Rica, about 15 km south of the Nicaraguan border, which conserves the highest percentage of forest cover, and contains the most valuable habitat for biodiversity within the corridor (Chassot & Monge, 2006). Our study area was a primary forest within the MNWLR (10o27'05.7''N, 84o16'24.32W.) and located within the private lands of Laguna del Lagarto Lodge that has not been harvested in at least 80 years, if at all, and contains regions of the forest that are dominated by P. *macroloba* (PM-D) and regions with few to none to these trees (PM-L).

Fig. 1. Location of the Maquenque National Wildlife Refuge (MNWLR) in Northern Costa Rica (map is from Dr. Olivier Chassot, Scientific Director, Centro Cientifico Tropical, E-mail: ochassot@cct.or.cr).

During July of 2006 and 2007, as part of a Tropical Ecology research course, a preliminary study of these forests was conducted in which microbial activity-linked measurements were taken in ten 5 m x 5 m plots in regions of these forests that were visibly dominated by *P. macroloba,* with either tree trunks and/or canopy coverage from these trees being within the plots, and ten plots in regions that had no trunks or canopy coverage within the plots—thus, these plots were considered to be not dominated by *P. macroloba*. In this unpublished preliminary work, we found that soil respiration (808±372 vs. 554± 323 µg CO_2/g/24h), soil total mineral N (2.92±0.61 vs.1.73± 0.27mg mineral N/g), the percent of the mineral N as nitrate (31% ±22% vs. 19% ± 9%), were all greater, and the qCO_2 (8±6 vs. 4±3) as an indicator of C use efficiency (lower value = greater efficiency)/g/24h) was less in the *P. macroloba*-dominant forests. However, the soil C biomass levels were greater in the forest

regions not dominated by *P. macroloba* (137±48 vs.101± 41 µg C/g soil). These results suggested that *P. macroloba* may be playing an interesting role in the C and N dynamics in these soils, warranting more work.

Based on this work, studies were conducted in the summers (June and July) from 2008- 2011 in these forested areas. Four 15 x 20 m plots were established in *P. macroloba*-dominant (PM-D) and limited (PM-L) regions, using the definitions above. during the summer of 2008, 2009 and 2010 (n = 12), these plots were assessed for the amounts of soil dissolved organic C (DOC), total mineral N (TMN), the rates of ammonium oxidation, Total N, soil C biomass (Cmic), soil N biomass (Nmic), and the abundance of various microbial groups were determined. In 2010 (n = 4), we also determined the rates of Cmic and Nmic development, soil laccase activity, the structural characteristics of *P. macroloba* and the understory vegetation community composition in these forests.

2.2 Soil collection and carbon, nitrogen, phosphorus, and laccase analysis

Each year, using sterile technique, we collected 25 randomly located 2 cm wide x 15 cm deep soil cores within each plot, over two consecutive days, composited the soil by plot, and sieved it at field moist conditions through 8 mm mesh. Percent saturation, pH, and bulk density were determined at 10 randomly located sites within each plot. All nutrient and microbial activity data presented have been adjusted for dry weight and bulk density of the soil.

The amount of total mineral N (TMN) as the total amount of ammonium (NH_4-N) and nitrate (NO_3-N) were determined following 2M KCl extraction of 10 g of soil (Alef and Nannapieri, 1995) using the 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). Nitrification rates were measured as the difference in nitrate levels of unincubated samples and samples incubated for 4 days. The microbial biomass N (Nmic) was determined as the difference in Total N in chloroform fumigated vs. unfumigated soils, using the potassium thiosulfate oxidation methods of Jiménez *et* al (2008); and the rates of Nmic development were determined as the difference in Nmic from unincubated samples and samples incubated for 4 days.

Several indicators of decomposition and potential C-sequestration activities, and organic C-use efficiency were examined. The soil dissolved organic C (DOC) was determined by the Walkley-Black rapid dichromate procedure, modified by (Nelson and Sommers, 1996). The soil biomass C (Cmic) determined as the difference between the DOC levels in chloroform-fumigated and unfumigated (total DOC) soil samples using the methods of Anderson and Ingram (1993). The rates of Cmic development were determined as the difference in Cmic from unincubated samples and those incubated over 4 days. The C use efficiency (Cmic/DOC) was calculated by the methods of Moscatelli *et al.* (2005) to suggest the efficiency at which the soil community uses organic C and incorporates it into the biomass. Phosphorous (P) content was measured following Bray 1 extraction from 2 g of soil using the molybdenate reduction method (Method # 8048) and the HACH DR 2700 system. From these data the C to N and N to P ratios were calculated. The phenol oxidase assay (Saiya-

Cork et al. 2002) was also determined to measure the rate of laccase activity over time as an indicator of the fungal-associated degradation of lignin (Sinsabaugh 2010).

2.3 Abundance of soil microbial groups

Soil microbial community DNA was extracted from three 0.3-g replicate samples of pooled soil using the Power Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, Catalog #: 12888), and the DNA extracts from each replicate then pooled. The soil DNA concentration was determined by agarose gel electrophoresis, using the BioRad Precision Molecular Marker Mass Standard and GeneTools software. The percent relative abundance (%RA) of a variety of microbial groups important in the C and N cycles were estimated by qPCR analysis. The %RA was determined for bacterial 16s rRNA, fungal 18s rRNA, fungal ITS regions, Types 1 and 2 methanotroph 16s rRNA, Proteobacteria AOB, and Archaeal 16s rRNA using the published PCR primers and reaction conditions of Martin-Laurent et al. (2001), Smit et al. (1999), Gardes et al. (1993), Chen et al. (2007), Webster et al. (2002), Kemnitz et al. (2005), respectively, and a MJ Research Opticon 1 Real Time Thermal Cycler. Each PCR product was assessed by agarose electrophoresis to confirm the presence of the correct size DNA bands. For the qPCR analyses, the fluorescence values were determined for sample DNA and for known concentrations of cloned control target DNA (7 to 30 ng/µL of cloned target gene DNA with sequences confirmed in GenBank). These values were used to compare the threshold cycle (C_t) for sample DNA to the C_t of the positive control DNA and then to calculate the abundance of the different target gene DNA concentrations in relation to the total abundance for all target genes to result in the %RA calculations.

2.4 Vegetation assessment

During the summer of 2010, an assessment of the vegetation community was conducted. Within each of the plots, five 1m² quadrats were randomly placed to sample understory vegetation. Within each quadrat, percent cover and density were calculated for all plant species occupying over 1% of the area and the total percent cover of the understory vegetation and overstory was estimated for the 1m². Aboveground herbaceous and shrub biomass was calculated by clipping all above-ground vegetation in the quadrats down to the soil level, bringing it back to the lab, drying in an oven and weighing the material. Species of plants occurring in the quadrats were identified in the field if possible, or pressed and brought back to be identified by local experts.

The diversity of the understory vegetation was categorized also into N-fixing and non-N-fixing vegetation groups. The richness and abundance of N-fixing species were calculated in each of the five quadrats in each plot and summed to get these measurements per 5m². We used the number of observed species per 5m² as an index of species richness and stem density as a measure of abundance and to suggest the diversity of N-fixing plants between the sites.

2.5 Statistical analyses

For data analysis, forest type (PM-D stands vs. PM-L stands) was the independent variable. Dependent variables that were compared between forests using SPSS (α=0.1) on the plot

scale included all nutrient, biomass, and microbial metrics; the number of *P. macrloba* seedlings, *P. macroloba* seedling height (in classes of <1m and >1m), *P. macroloba* tree diameter at breast height and heights of the trees). Dependent variables from the quadrat-scales included the percent cover of total overstory, total understory, percent cover for each species, and density of each woody species in the quadrats. Additional analyses were performed comparing the sum of the 5 quadrats in each plot for biomass, the total density of legumes (including *P. macroloba*) rooted in the quadrats, the species richness of legumes, and the total percent cover of legumes in each quadrat.

A weight of evidence statistical approach was used to compare differences in the mean values of all metrics determined from the two habitats. The mean and standard deviations were determined for each metric, and the percent differences (PD), T-test *p* values, and the Hedge'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 a combined approach of T-test *p* values ≤ 0.1, PD ≥ 20%, and Hedge's *d* values ≥ 0.7 (>0.7 is considered a large effect size difference) as a weight of evidence to define biologically important differences in the mean values for this project.

3. Results

3.1 Soil nutrient, biomass and microbial analyses

There were many biologically important differences (defined as T-test *p* values ≤ 0.1, PD ≥ 20%, and Hedge's *d* values ≥ 0.7) found in the soils between the *Pentaclethra macroloba*-dominant (PM-D) and *P. macroloba*-limited (PM-L) stands (Table 1). The PM-D stand soils had greater amounts of inorganic nutrients in general. Specifically, these soils had greater levels of phosphate, TMN, and percent of inorganic N as NO_3, nitrification rates, and N-biomass than found in the PM-L soils, which had a greater rate of N-biomass development and more total N. Consistent with this was the finding that there was a greater amount of bacterial DNA, in general, and AOB DNA specifically in the PM-D stand soils. In addition, there was a much lower N:P ratio found in the PM-D than PM-L soils.

There was a greater amount of DOC and soil C-biomass found in the PM-L stands, which also had greater levels of the indicator of C-use efficiency (Cmic/DOC), and a greater C to N ratio (Table 1). The rate of C-biomass development was somewhat greater in the PM-L stand soils, but did not reach our defined critical level of biological importance (*p* = 0.112, *d* = 0.68). However it does suggest a trend toward a more rapid C-biomass development rate. Consistent with these data was the greater amount of fungal rRNA gene and fungal ITS DNA. The laccase activity for lignin degradation, an indicator of more complex organic C decomposition (Guggenberger et al. 1994, 1995, Guggenberger and Zech 1999, de Boer et al. 2005, Bradford et al. 2008), was also greater in the PM-L stand soils (Table 1). The amount of Archaea and Methylotroph Type 2 DNA was about equally high in both soil types, but the Methylotroph Type 1 was greater in the PM-D soils. This suggests that the Archaea and Type 2 Methylotrophs are playing some kind of important role in the C and N cycle activities in both soils types.

Metric	*P. macroloba-Limited*		*P. macroloba-Dominant*		% Difference	T-Test *p* Value	Hedge's *d* value	Standard Error of E.S.
	mean	SD	mean	SD				
Nitrification (mg/CC/24h)	0.194	0.034	0.253	0.027	30.40	0.00200	1.53	0.57
Total N(mg/cc)	1.93	0.41	1.54	0.42	20.10	0.08200	0.75	0.52
TMN (mg/cc)	2.21	0.62	6.51	0.66	194.57	<0.0001	5.36	1.07
% as NO3	16.73	9.33	33.48	18.41	100.12	0.03767	0.92	0.53
Phosphorus(μg/cc)	9.95	5.64	29.24	14.03	193.90	0.00300	1.43	0.56
N:P	0.23	0.14	0.04	0.01	82.6	0.0018	1.53	0.57
Nmic (mg/cc)	0.19	0.06	0.7	0.29	268.42	0.00025	1.94	0.61
Nmic(mg/cc/24h)	3.22	2.47	1.24	0.96	61.49	0.05301	0.84	0.52
DOC (μg/cc)	671	141	523	108	22.10	0.03400	1.95	0.61
Cmic (μg/cc)	107	23	53	17	50.47	0.00010	2.13	0.63
Cmic(μg/cc/24h)	53	21	36	19	32.08	0.11200	0.68	0.51
Cmic:DOC	0.16	0.042	0.126	0.034	21.25	0.09700	0.63	0.51
C:N	0.39	0.09	0.29	0.12	25.64	0.08000	0.75	0.52
Laccase (OD/h)	0.0049	0.002	0.0013	0.001	73.47	0.00450	1.82	0.59
% RA UB rRNA	10.9	1.2	19.9	2.12	82.57	0.00000	4.17	0.89
%RA UF rRNA	18.35	9.83	7.21	3.12	60.71	0.00856	1.22	0.54
%RA Fungal ITS	16.1	6.41	9.01	4.12	44.04	0.01972	1.05	0.53
%RA Arch	16.57	7	16.59	5.12	0.12	0.99489	0.00	0.50
%RA Meth1	9.49	8.21	16.76	6.12	76.61	0.06433	0.80	0.52
%RA Meth2	26.8	9.01	27.22	7.12	1.57	0.91908	0.04	0.50
%RA AOB	1.79	0.84	3.45	0.48	92.74	0.00026	1.94	0.61

Table 1. A comparison of the mean levels (± standard deviation) of carbon, nitrogen, biomass, and microbial group-related metrics from soils within a primary forest in regions dominated by *Pentaclethra macroloba* (PM-D) and in regions with little to no *P. macroloba*: Presented are differences between the PM-L and PM-D regions in the rates of Nitrification (mg/CC/24h), the amount of Total N(mg/cc), the total mineral nitrogen levels, or TMN (mg/cc), the percent of the nitrogen that is present as nitrate (% as NO3), the levels of Phosphorus (μg/cc), the ratio of N:P, the amount of soil biomass nitrogen or Nmic (mg/cc), the rate at which the Nmic develops (mg/cc/24h), the amount of dissolved organic carbon, or DOC (μg/cc), the amount of soil biomass carbon or Cmic (μg/cc), the rate at which Cmic develops (μg/cc/24h), the ratio of Cmic:DOC, the ratio of C:N, and the amount of laccase activity (OD/h). Also presented are the percent mean relative abundance (% RA) values (± standard deviation) of DNA using qPCR targeted the total bacterial 16S rRNA (%RA UB rRNA), total fungal 18S rRNA (%RA UF rRNA), the fungal internal transcribed space region (%RA Fungal ITS), Types 1 and 2 Methylotrophs 16S rRNA (%RA Meth 1 and 2), Archaea 16S rRNA (%RA Archaea), and ammonium oxidizing bacteria 16S rRNA (%RA AOB). Data were analyzed to determine biologically important differences between the means, which was defined as mean differences having T-test *p* values ≤ 0.1, % difference levels ≥ 20%, and Hedge's *d* values ≥ 0.7 (>0.7 is considered a large effect size difference) as a weight of evidence.

3.2 Vegetation analyses

There were also differences in mean values of the metrics describing the vegetation structure between the two habitats that met our definition of being biologically important (Table 2). The *P. macroloba* density was greater in the PM-D stands (p=0.014), as was the percent cover of the overstory due to *P. macroloba* (p=0.005). However, the plots seemed to be similar in overall vegetation structure as this study did not find strong evidence of a difference in biomass (p=0.208), the percent cover of shrubs in the quadrats (p=0.846) or the total overstory cover, of all tree species combined (p=0.306) between the PM-D and PM-L plots. Thus, although there are similarities in structure, it appears that different species are fulfilling these roles. As well, there were a total of 51 plant species were identified in the PM-D forest sampling quadrats and 39 were identified in the PM-L quadrats. Therefore, the PM-D stand overall plant richness was greater than in the PM-L (p=0.060). There was a total of nine N-fixing plant species identified in the PM-D stands, and six such species in the PM-L stands, resulting in a greater richness index of N-fixing plants in the PM-D stands (p=0.094). There was also a greater percent of *P. macroloba* seedlings in the PM-D stands (p=0.074).

Metric	P. macroloba-Limited		P. macroloba-Dominant		% Difference	T-Test p Value	Hedge's d value	Standard Error of E.S.
	mean	SD	mean	SD				
Total Understory S	18.25	2.29	25.75	2.29	29.13	0.06000	2.61	0.96
N-Fixing Veg S	1.75	0.48	3	0.41	41.67	0.00745	2.23	0.90
biomass	442	93	830	542	46.75	0.20790	0.80	0.73
PM density	0.25	0.5	5.25	2.9	95.24	0.01453	1.92	0.85
% PM Overstory	16.25	10.9	80.5	27.8	79.81	0.00507	2.43	0.93
Total Overstory	96.2	6.8	100	0	3.80	0.30646	0.63	0.72
%Shrub Cover	68.6	22.7	65.4	21.8	-4.89	0.84558	0.11	0.71
%PM as Seedlings	1.5	1.9	19.8	16.8	92.42	0.07359	1.22	0.77

Table 2. A comparison of the vegetation characteristics in tropical lowland forested regions dominated by *Pentaclethra macroloba* (PM-D) and in regions with little to no *P. macroloba* within the Maquenque National Wildlife Refuge in the Northern Zone of Costa Rica: Presented are the differences between the PM-L and PM-D regions in the mean levels (± standard deviation) of the total richness of the understory vegetation (Total Understory S), the richness of the nitrogen-fixing vegetation (N-Fixing Veg S), the biomass of the vegetation (biomass), the *P. macroloba* density (PM density), the percent of the overstory that is from *P. macroloba* (% PM Overstory), the total amount of the overstory vegetation (Total Overstory), the percent of the understory that is shrub cover (%Shrub Cover), and the percent of the seedlings that are *P. macroloba* (%PM as Seedlings). Data were analyzed to determine biologically important differences between the means, which was defined as mean differences having T-test p values ≤ 0.1, % difference levels ≥ 20%, and Hedge's d values ≥ 0.7 (>0.7 is considered a large effect size difference) as a weight of evidence.

4. Discussion

The data from this work suggest that PM-D stands are very important in recuperating and maintaining the N levels in these soils. They also appear to be facilitating the growth of more N-fixing understory vegetation, more young growth of plants, and an overall greater vegetation richness in the PM-D stands, suggesting a vegetation structure that is less homogenous (and perhaps less stable) than in the PM-L stands. Both the N-fixing understory and the *P. macroloba* trees in the PM-D stands are providing the needed inorganic N to stimulate development of a more complex organic C and N composition somewhat distance from these trees. This is likely a dynamic situation that requires a balance between N cycle bacterial-associated stimulation and fungal stimulation and inhibition in order to generate what appears to be a greater potential for C and N sequestration in the PM-L soils. It appears that the structure of the PM-L stands is more similar to an older, more established forest with a more stable and somewhat less diverse vegetation, yet with high levels of biomass, but these parts of the forest are clearly dependent on the PM-D stands and the nutrients they provide.

There is a complex relationship between increases in plant-derived carbohydrates, lignin, celluloses and other more recalcitrant organic compounds, soil inorganic N, increased soil complexity (Guggenberger et al. 1994, Zech and Kögel-Knaber 1994; Bradford et al. 2008) and soil biomass development (Anderson and Domsch 1989; Brookes 1995; Anderson 2003; He et al. 2003; Moscatelli et al. 2005), and the clear differences in vegetation structure between the PM-D and PM-L stands appear to be playing a role in the dynamics of this relationship in these forests. Increased levels of soil inorganic N stimulate rhizodeposition and increased production of more labile root-derived carbohydrates used to enhance the bacterial community, although increases in inorganic N also inhibit fungi (Bittman *et al.* 2005, de Vries *et al.* 2007). Plant lignins and other more recalcitrant organic compunds select for Basidiomycota and other fungi that degrade the lignin, celluloses, etc (Sinsabaugh 2010). Both of these types of microbial-directed processes enhance the soil organic matter complexity, stimulating complex microbial activities associated with decomposition, soil respiration, and mineralization of organic matter, followed by an increase in organic compounds and soil biomass (Andrews and Harris 1986; Powlson et al. 1987; Guggenberger et al. 1994, 1995; Zhang and Zak 1995; Arunachalam et al. 1997; Eaton 2001; Padmanabhan et al. 2003; de Boer et al. 2005; Schwendenmann and Veldkamp 2006; Fierer et al. 2007; Bradford et al. 2008), a more efficient use of the soil organic matter, and more organic C and N available to the foodweb (Anderson and Domsch 1989; Brookes 1995; Anderson 2003; He et al. 2003; Moscatelli et al. 2005).

The N-cycle data from the current study suggests that in the PM-D stands, there was a greater amount of microbial activity involved in the development of inorganic nutrients, typically associated more with the bacterial than fungal communities (e.g., Moore and de Ruiter 1991; Lovell et al. 1995). This is likely due to the greater amount of N-fixing vegetation in the PM-D stands, including the greater amount of *P.macroloba*. This would explain the greater amount of N cycle activity, greater amount of inorganic N and Nmic, and bacterial and AOB DNA in the PM-D soils, suggesting a greater amount of N-fixation and ammonium oxidation was occurring there. Fungi, and especially some groups like the Basidiomycota, are known to harvest organic C and N, moving it into the biomass and making it available to the other parts of the forest soil food web (e.g. Talbot et al 2008).

However, increases of inorganic N in soils have been shown to suppress fungal biomass development (Bittman *et al.* 2005, de Vries *et al.* 2007) and the decomposition of lignin and lignaceous material. The latter occurs through inhibition of lignolytic enzyme (i.e., laccase) synthesis (Worrall *et al.* 1997, Carreiro *et al.* 2000, Sinsabaugh *et al.* 2002 and 2004, Knorr 2005, Waldrop and Zak 2006) and/or by reacting with lignin degradation products to form more recalcitrant compounds (Dijkstra *et al.* 2004, Hobbie 2008). This is what appears to be happening in the PM-D soils. The PM-L stands have fewer N-fixing plants and *P. macroloba,* but still and equally strong overall vegetation community, which is associated with the lower levels of inorganic N, and greater amounts of organic N (as part of the total N measurement), N-biomass, and rates of development of the N-biomass in the PM-L stands, along with the greater amounts of fungal DNA and laccase activity. This all suggests that the PM-L stands are facilitating development of a more fungal-dominant soil microbial community, and the PM-D stands, due to the inorganic N, are facilitating the development of a more bacterial-dominant soil microbial community. The fungal dominant community in the PM-L stand soils would result in the production of more N and C biomass and stable forms of organic C and N material in these soils than in the PM-D soils.

The C cycle data from this study showed there was a greater amount of soil DOC, amount and rate of development of C biomass and laccase activity, and more fungal DNA in the PM-L soils. The greater amount of laccase activity, that is associated with lignin degradation, is an indicator of an enhanced Basidiomycota fungal population (see de Boer *et al.* 2005 for a review) and a more complex organic C decomposition (Guggenberger et al. 1994, 1995, Guggenberger and Zech 1999, de Boer et al. 2005, Bradford et al. 2008). Again, this is associated with a more homogenous vegetation community in the PM-L stands that have less N-fixing vegetation. The increased efficiency of C use in the PM-L stand soils, as measured by the ratio of C biomass to the DOC, is also an indicator of an enhanced soil fungal population. An increase in this metric suggests there is an increase in the amount of organic C being made available for the microbial community and for transfer up the food web (Anderson & Domsch 1989, Brookes 1995, Anderson 2003, Moscatelli *et al.* 2005), and usually is associated with a shift towards fungal dominance (Anderson 2003). An increase in this ratio often occurs as a result of an increasing fungal biomass in comparison to a relatively static bacterial biomass (Ohtonen *et al.* 1999, Van der Wal *et al.* 2006). As well, due to the inhibition of fungi by inorganic N and the increase in organic C often associated with an increase in fungal biomass, an increase in the C to N ratios is also an indicator of a fungal-dominant soil biota (Anderson, 2003).

As stated in the Introduction, fungi are thought to decompose organic substrates more efficiently than bacteria (Holland and Coleman, 1987; Griffith and Bardgett, 2000), leaving behind more complex fungal biomass organic residues than bacterial residues, thus increasing the amount of recalcitrant organic matter and an enhanced DOC (e.g., Malik and Haider, 1982; Guggenberger et al., 1999; Sinsabaugh 2010). The saprotrophic fungi play major roles in the decomposition of dead plant material, particularly cellulose and lignin in the litter and organic soil horizons (Luis et al., 2004; O'Brien et al., 2005), and are thought to be very diverse in lowland tropical soils (Bills and Polishook, 1994). Mycorrhizal fungi are mutuallistic symbionts of plants (Malloch et al., 1980), and are especially abundant in the O and A soil horizons (Luis et al., 2004; O'Brien et al., 2005) and are now being shown to play important roles in both providing materials to plants and in organic C and N harvesting from surrounding soil regions (Talbot et al 2008). The observations from the soil analyses in

our study were consistent with a more fungal-dominant soil ecosystem in the PM-L soils. The greater amount of N-fixing vegetation enhances the bacterial community in the soils associated with the inorganic N-cycle processes, resulting in greater amounts of inorganic N which would somewhat inhibit the Basidiomycota and/or other laccase producing fungi. In addition, it is also possible that the vegetation in the PM-L stands are releasing materials that are more conducive to establishing a more dominant fungal community. This should be examined in the future. Nonetheless, it is evident that the N-fixing vegetation in the PM-D stands are both inhibiting the soil fungi, and providing what is needed by them for use at some distance to the trees. This is a critical dynamic that must be balanced in order to enhance the fungal community in these older primary forests to allow them to function properly.

This study also showed that Archaea and Methylotrophs may be important in both the PM-D and PM-L stands in enhancing the organic C and N. The methylotrophs play an important role in soils by using methane as their sole energy source, converting it into forms of organic carbon (C) that can be utilized up the food web and are indicators of a complex soil system (Bastyiken et al 2003, Bull et al 2000, Murase and Frenzel 2007, Mancinelli 1995, Hanson and Hanson 1996, Whalen et al 1990), and that some are capable of N fixation (Chu and Alvarez-Cohen 1999, Auman et al. 2001). The role of the archaeal community in forest soils is much less clear, although some of their various functions are known or have been proposed. The terrestrial representatives of the Crenarchaeote Group 1.1b (Chaben et al 2006) have been found in a wide variety of soil types (Ochsenreiter et al 2003, Sliwinski and Goodman 2004). Many Crenarchaeotes have been found associated with a wide variety of plant species within the rhizosphere (Simon et al 2000 and 2005; Chelius and Triplett 2001; Sliwinski and Goodman 2004), and are thought to be aerobic heterotrophs (Rutz and Kieft 2004). More recently, ammonia-oxidizing archaea (AOA) within the *Crenarchaeota* group have also been found to play this important role in soils (Hallam *et al.* 2006; Schleper *et al.* 2005; Treusch *et al.* 2005). In some cases, the AOA have been shown to be more numerically dominant over the AOB group in terrestrial soils (Adair and Schwartz 2008; Chen *et al.* 2008; He *et al.* 2007), although this is still controversial as it has been pointed out that abundance may not correlate with contribution (i.e., Wessen et al 2010). Given both the wide range of plant species the Crenarchaeota have been associated with, and that they are known AO microbes, it is not a surprise that they are fairly equally distributed in these two habitats. Although they are clearly important in developing appropriate amounts and composition of C and N materials in soils, there is a significant gap in the knowledge about how these microbes interact or compete with plants and other soil members of the microbial communities for C and N, and how they change during plant growth or changing plant species during succession in tropical forest ecosystems.

The rate and amount of soil N-fixation activity is associated with the development of the organic C and N composition in soils, and is also known to be regulated by a feedback inhibition mechanism that is activated in the presence of higher N:P ratios or lower concentrations of P (Eisele *et al.* 1989, Smith 1992, Israel 1993, Almeida *et al.* 2000, Schulze 2004, Pons *et al.* 2007, Reed *et al.* 2007). This is consistent with the results of our work in which there were somewhat greater concentrations of total N and much lower concentrations of P in the PM-L soils, resulting in higher N:P ratios (i.e., P limited), greater soil C biomass, greater rates of both C and N biomass production, and a more fungal-dominant microbial community in the PM-L soils.

Changes in N:P ratios have been associated with increases in microbial activity and biomass, N and P cycle processes, and microbial community structure (Leahy and Colwell 1990, Smith 1992, Smith *et al.* 1998, Cleveland *et al.* 2004, Cleveland and Townsend 2006, Allison *et al* 2007, Cruz *et al.* 2008). It has also been shown that well-established tropical forest soils tend towards more P than nitrogen limitation (Vitousek & Farrington 1997, Sollins 1998, Hedin *et al.* 2003), resulting in higher N:P ratios, in part due to the extensive amount of nitrogen fixation that occurs (Vitousek & Howarth 1991, Cleveland *et al.* 1999, Galloway *et al.* 2004), and as a result of the decomposition of greater amounts of forest litter and vegetation tissues (see McGroddy *et al.* 2004 for a review). Our results lead us to pose that the differences in the above-ground vegetation, especially the N-fixing species, in the PM-D and PM-L stands, and the subsequent differences in stimulation of either a more bacterial or fungal-dominant biota, are altering the ratios of N to P and are, thus, helping to drive the microbial community function in these soils. This results from changes in the N-fixation and ammonium oxidation, and the abundance of the associated bacterial groups responsible for these processes. As well, lower concentrations of inorganic N facilitate development of fungal populations, in particular, fungi such as Basidiomycota and others associated with an increase in laccase activity—due to the reduction in inorganic N-associated inhibition of these groups.

The increased fungal dominance and laccase activity observed in the current study occurs along with an increase in DOC, total N, rates of incorporation of N into the biomass, rates of C biomass development, and a more efficient use of organic C and N suggest that the PM-L soils have a more fungal-dominant biota. These differences in patterns created by the structure of the *P. macroloba* forest stands have very important implications for P, N and C cycle dynamics, soil organic matter development, lignin degradation and other types of more complex organic matter decomposition, and C and N biomass development in these soils, thus C and N sequestration. To our knowledge, this is the first time that N:P ratios in old growth tropical forests have been examined in relation to differences in above ground vegetation and below ground microbial community composition. Our data show that as the below-ground N:P increases (P is more limited) the below ground biomass increases, and also that this also occurs with as the C:N ratio increases. Moreover, this is occurring in conjunction with decreased amount and diversity of N-fixing vegetation in the PM-L stands, further from the regions dominated by *P. macroloba.* If confirmed, this model has the potential to account for changes in the below ground microbial-related biomass development due to differences in environmental or management conditions that affect the soil N and P, with potential links to C and N sequestration predictions.

5. Conclusion

In these older primary tropical forests, the PM-D stands have a greater amount of and diversity of N-fixing vegetation than the PM-L stands, appearing similar to a forest in a younger stage of succession. Associated with this is a more bacterial-dominant soil biotic community than the PM-L soils, with greater amounts and rates of production of inorganic nutrients, but slower rates of development of below ground C and N biomass, resulting in less DOC and total N than the soils from the PM-L regions. The PM-L soils have far less N-fixing vegetation, yet a strong and healthy vegetation community with similar amounts of coverage and biomass as the PM-D forests, but with different non-N-fixing species involved. Associated with this were less inorganic N, greater amounts of DOC and total N, and greater amounts and rates of production of C and N biomass. This appears to be due to the greater abundance

of fungi, linked to their capcity for more complex decomposition, indicated by the greater amounts of laccase activity in the PM-L soils. Thus, the PM-L stand soils are more fungal-dominant, likely resulting in a more complex soil organic C composition, and a more complex microbial community that is making more efficient use of the nutrients provided by the nearby PM-D regions. This probably results in development of more C and N biomass in the PM-L soils, and potentially a greater amount of C and N sequestration in these soils.

The conclusions posed from this work are enticing; however, they also illustrate the tremendous need for much more work to clarify many questions concerning the effect of *P. macroloba* on the soil community and nutrient dynamics and the vegetation community. Perhaps the most striking results to us were how distinct the two microbial and vegetation communities were in the PM-D and PM-L stands. The observed relationships between the C:N and N:P ratios, the evidence for enhanced fungal dominance and soil complexity, and the changes in the vegetation communities warrant more work in the future, and represent major areas of study that should be targeted in order to better understand how this N-fixing tree, that is so critical to these tropical forests, functions to stimulate soil and vegetation ecosystem development and maintenance of a stable and healthy ecosystem.

There are many other questions that remain unanswered concerning the role of this important member of the Fabaceae in these forest ecosystems, which should also serve as targets for future work. How does PM affect the succession of soils in secondary forest regeneration? At what point, over what time frame, and under what conditions does PM enter into a damaged/cleared forest as an early successional tree to recuperate the N in the soil system, re-establish the vegetation, and stimulate development of a "normal" level of both soil and above ground vegetation population dynamics, including C and N sequestration? What conditions are needed for the trees to begin to stimulate the production of inorganic N that stimulates the initial pulse of bacterial activity, which is then needed to stimulate the development of the more simple, then followed by the more complex fungal populations and their associated activities. At what point does the fungal population begin to dominate and how far from the individual trees does this happen—thus, how far from the trees is the stimulating effect of the increased N on the fungal populations, which is balanced out by the inhibitory effect of the inorganic N on fungal populations and laccase activity? It appears as if the PM is important in driving the bacterial-associated N-cycle activity and the provision of inorganic N in these soils, and that this is being used in the immediate area by the bacterial community. However, at some distance away from the PM-D stands (How far?), the fungal inhibition by inorganic N is reduced, and the fungal community is being stimulated and is making use of this inorganic N to build up and maintain their populations and to generate and distribute organic forms of both N and C into the system. Also, if there is an increase in complexity of organic C decomposition occurring at some distance to the trees, is there production of more stable forms of organic C being produced away from the PM-D regions, and if so, at what distance from the PM-D region does this occur, and at what density of PM trees does the this occur?

These are important questions for land managers in the region as there are a variety of strategies of forest regeneration occurring in the region, and it is critical for them to know what the end result forest target should be, so that they know when a secondary forest is re-established as a healthy stable ecosystem, and when might it be least damaging to the ecosystem to selectively re-harvest certain species of trees in that secondary forest. The answers to these questions are also important for developing a more complete

understanding of the biotic and abiotic factors that drive the C and N biogeochemical processes in tropical forest ecosystems. More studies should be conducted which examine the impact that critical ecosystem species (keystone perhaps?) have on forest ecosystems, such as an early successional N-fixing tree like *P. macroloba*, as they can provide important information to begin to address these and many more questions concerning the C and N cycle dynamic processes in tropical forest ecosystems, and how they might be affected by anthropogenic changes in the environment.

6. Acknowlegements

7. References

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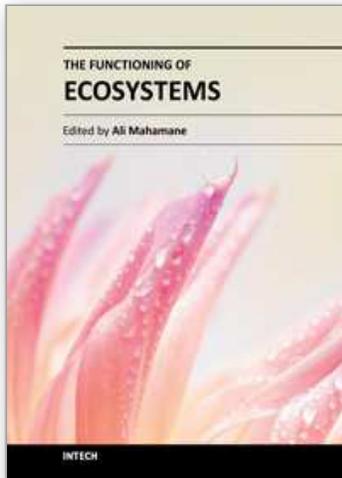
The Functioning of Ecosystems

Edited by Prof. Mahamane Ali

The ecosystems present a great diversity worldwide and use various functionalities according to ecologic regions. In this new context of variability and climatic changes, these ecosystems undergo notable modifications amplified by domestic uses of which it was subjected to. Indeed the ecosystems render diverse services to humanity from their composition and structure but the tolerable levels are unknown. The preservation of these ecosystemic services needs a clear understanding of their complexity. The role of the research is not only to characterise the ecosystems but also to clearly define the tolerable usage levels. Their characterisation proves to be important not only for the local populations that use it but also for the conservation of biodiversity. Hence, the measurement, management and protection of ecosystems need innovative and diverse methods. For all these reasons, the aim of this book is to bring out a general view on the biogeochemical cycles, the ecological imprints, the mathematical models and theories applicable to many situations.

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