Tropical Ecology **53**(2): 197-206, 2012 ISSN 0564-3295

www.tropecol.com

Interactions between populations of *Rhizobium*, Methanotrophs and Archaea in two different lowland tropical forest soil communities

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Abstract: Bacteria in the genus *Rhizobium*, methanogenic bacteria and Archaea are important in the terrestrial carbon (C) and nitrogen (N) cycle dynamics and the sequestration of C and N into the soil biomass. A better understanding of the functions of these microbial groups could provide some clarity on the impact of different land development practices and a changing climate on soil ecosystems. The community structure of these three groups of soil microbes was compared between a secondary forest and a forest dominated by the leguminous tree *Pentaclethra macroloba* within a Costa Rican rainforest. The secondary forest soils had a greater total microbial biomass and efficiency of C utilization, greater relative abundance of methanotrophs and Archaea 16s rDNA, and greater overall microbial diversity, whereas the relative abundance of *Rhizobium* was greater in the *Pentaclethra macroloba*-dominant forest soil. The data suggest that rhizobia, methanotrophs and Archaea are involved in a complex interplay that affects the C and N cycle dynamics.

Resumen: Las bacterias del género *Rhizobium*, las bacterias metanogénicas y los Archaea son importantes en la dinámica de los ciclos del carbono (C) y nitrógeno (N) terrestres y en el secuestro de C y N en la biomasa del suelo. Una mejor comprensión de las funciones de estos grupos microbianos podría brindar claridad sobre el impacto de las diferentes prácticas de uso del suelo y del cambio climático sobre los ecosistemas edáficos. La estructura de la comunidad de estos tres grupos de microbios del suelo fue comparada entre un bosque secundario y un bosque dominado por la leguminosa arbórea Pentaclethra macroloba en un bosque lluvioso de Costa Rica. Los suelos del bosque secundario tuvieron una biomasa microbiana total mayor y una mayor eficiencia en la utilización de C, una mayor abundancia relativa de ADNr 16s de los metanotrofos y los Archaea, y en general una mayor diversidad microbiana, mientras que la abundancia relativa de *Rhizobium* fue mayor en el suelo del bosque dominado por Pentaclethra macroloba. Los datos sugieren que los rizobios, los metanotrofos y los Archaea están involucrados en una interacción compleja que afecta los ciclos dinámicos del C y el N.

Resumo: Bactérias do género *Rhizobium*, metanogénicas e Archaea são importantes nas dinâmicas do ciclo de nutrientes do carbono terrestre (C) e do azoto (N) e na sequestração do C e N na biomassa do solo. Uma melhor compreensão das funções destes grupos microbianos pode proporcionar uma melhor clarificação quanto ao impacte das diferentes práticas de desenvolvimento da terra e da mudança climática nos ecossistemas do solo. A estrutura de

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comunidade destes três grupos de micróbios do solo foi comparada entre a floresta secundária e a floresta dominada pela Pentaclethra macroloba, uma espécie arbórea leguminosa na floresta de chuvas da Costa Rica. Os solos da floresta secundária apresentaram uma maior biomassa microbiana total e mais eficiência na utilização do C, maior abundância relativa de metanotrofos e Archaea 16s rDNA, bem como maior diversidade microbiana global, enquanto a abundância de Rhizobium foi maior nos solos florestais dominados pela Pentaclethra macroloba. Os dados sugerem que os *Rhizobium*, metanotrofos e Archaea estão envolvidos numa interação complexa que afecta as dinâmicas do ciclo do C e do N.

Key words: Archaea, microbial community structure, nitrogen fixing, *Pentaclethra macroloba, Rhizobium*, secondary forests.

Introduction

Rhizobia (Rosch *et al.* 2002; Widmer *et al.* 1999), methanotrophs (Hackl *et al.* 2004; Knief *et al.* 2003) and Archaea (Chaban *et al.* 2006; Kemnitz *et al.* 2007) are thought to play important roles in the carbon (C) and nitrogen (N) cycles in forested soils, yet little is known of their interactions within tropical forest habitats. Rhizobia are critical N-fixing bacteria that are both free-living and associated with root nodules of non-woody vegetation (Allen & Allen 1950; Fischer 1994; Freiberg *et al.* 1997; Mylona *et al.* 1995), such as that commonly found on the floor of tropical forests. They are thought to serve as important suppliers of inorganic N, which can stimulate rhizodeposition and increase production of more labile root-derived carbohydrates used by the bacterial community to enhance soil biomass development (Anderson 2003; Bradford *et al.* 2008; He *et al.* 2003; Moscatelli *et al.* 2005; Zech & Kögel-Knaber 1994).

The terrestrial representatives of the Archaeal Crenarchaeote Group 1.1b (Chaben *et al.* 2006) have been found in a wide variety of soil types under various conditions (Ochsenreiter *et al.* 2003; Sliwinski & Goodman 2004), with some properties not common for other Archaea. Some are thought to be aerobic heterotrophs important in enhancing soil organic C (Rutz & Kieft 2004; Simon *et al.* 2000; Simon *et al.* 2005; Wessén *et al.* 2010), and have been associated with a wide variety of plant species in the rhizosphere (Chelius & Triplett 2001; Simon *et al.* 2000, 2005; Sliwinski & Goodman 2004). Members of the Crenarchaeota have also been identified as ammonia-oxidizing microorganisms (AOM) important in converting ammonia to nitrite during nitrification, which is then converted to nitrate by nitrite-oxidizing bacteria, both processes being critical for providing N to forest ecosystems (Hallam *et al.* 2006; Schleper *et al.* 2005; Treusch *et al.* 2005). In fact, it has been suggested that, in some cases, the AO Archaea (AOA) can be numerically dominant over the AO bacteria (AOB) group in terrestrial soils (Adair & Schwartz 2008; Chen *et al.* 2008; He *et al.* 2007). Little is known about how the AOA populations affect - or are affected by - C and N inputs in forest ecosystems, especially in tropical forests, although it has been shown that such inputs into forest soils may select preferentially for heterotrophic AOA over chemoautotrophic AOB (He *et al.* 2007; Shen *et al.* 2008; Schauss *et al.* 2009). There is also developing evidence for the presence of N-fixing genes in different groups of Crenarchaeota, leading to the suggestion that some of these microbes may be involved in N-fixation (Chaban *et al.* 2006; Miyazaki *et al.* 2009; Pernthaler *et al.* 2008; Quaiser *et al.* 2002), although this has not been confirmed.

Methane is a trace gas in the atmosphere which, despite its short atmospheric residence time, is a potent greenhouse gas (Le Mer & Roger 2001; Wuebbles & Hayhoe 2002). Methanogenic Archaea have been found to be important sources of methane in peat bogs and soils in terrestrial environments (Bapteste *et al.* 2005; Garcia *et al.* 2000; Jones *et al.* 1987). Methanotrophic bacteria in forest use methane as their sole energy source, converting it into organic C, enhancing the soil organic C content in soils (Bastviken *et al.* 2003; Bull *et al.* 2000; Hanson & Hanson 1996; Mancinelli 1995; Murase & Frenzel 2007; Whalen *et al.* 1990). Some methanotrophs have also been shown to be capable of N fixation (Auman *et al.* 2001; Chu & Alvarez-Cohen 1999), enhancing the N compo-

nent of soils. Methanotrophic bacteria and methanogenic Archaea, thus interact in forest soils as components of the global C cycle.

These three groups of bacteria may play important roles in the global C and N cycles , and in C sequestration in forest soils, yet very little is known about the diversity and activity of these microbes in tropical forests. This knowledge can help in understanding the impact of land development practices, deforestation, and the effects of a changing climate in tropical areas (Butler & Laurance 2008; Malhi *et al.* 2008; Putz *et al.* 2000; Sader & Joyce 1988; Sánchez-Azofeifa *et al.* 2001; Schwartzman *et al.* 2000).

The present study was conducted in the lowland regions of the Northern Zone of Costa Rica, within a tropical forest that was cleared approximately 30 years ago, and allowed to regenerate into a mixed species secondary forest naturally with no interference. In regions of this forest near lagoons, there are large areas composed almost entirely of *Pentaclethra macroloba*, considered the dominant N-fixing tree in the forest (Hartshorn & Hammel 1994; Pons *et al.* 2007). This is the first study in Costa Rica, and perhaps all of Central America, to compare C and N nutrient metrics with the abundance and diversity of rhizobial, methanotrophic and archaeal microbial communities.

Materials and methods

Sampling sites

The forest sites used for this study were within the Maquenque National Wildlife Refuge (MNWLR), in the Northern zone of Costa Rica near the Nicaraguan border (10.7151 - 84.1697). In the early 1980s, this forest had been cleared and allowed to naturally regenerate resulting in a secondary forest typical of the region, in which the majority of the forest is not dominated by a single tree species, but patches within the forest are composed mainly of *Pentaclethra* trees (*Pentaclethra macroloba*), the dominant N-fixing tree in these forests. We selected four replicate plots within each of the *Pentaclethra macroloba*-dominant (*Pentaclethra* forest) and the mixed species (secondary forest) areas of the forest. The eight plots used were on a common trail, were of identical age, and were of the same slope and topography. The secondary forest plots had been previously established and were 30 x 30 m (Eaton, Pers. Comm.). The *Pentaclethra* forest plots of 20 x 15 m were established adjacent to the secondary forest plots. Sixteen randomly located 2 cm wide x 15 cm deep soil cores were collected within each plot, and composited for analysis over two consecutive days in July, 2009. Percent saturation and pH were determined at 10 randomly located sites within each subplot using a Kelway HB-2 Soil and pH meter (Wyckoff, NJ, USA). Four other 2 cm x 15 cm soil cores were collected from each subplot for bulk density analysis. Rocks, insects and plant matter were removed by sieving the samples at 5 mm. All data presented have been adjusted for dry weight and bulk density of the soil.

Nutrient, microbial biomass and respiration analyses

The amount of ammonium (NH_4-N), nitrate (NO_3-N), and total mineral N (TMN) were determined following 2M KCl extraction of 10 g of soil 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). The TMN was calculated as the amounts of NH_4-N and NO_3-N. Phosphate (PO_4) content was measured following Bray 1 extraction from 2 g of soil using the molybdate reduction method (HACH method # 8048) and the HACH DR 2700 system.

Microbial biomass C (C_{mic}) was determined by the fumigation-extraction method (Jenkinson 1988) as the difference between K_2SO4 extracted 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). Microbial metabolic quotients (qCO_2, as a ratio of CO_2 production from respiration/C_{mic}) and the percent ratio of microbial biomass to DOC (C_{mic}/DOC) were determined, in which lower qCO2 and greater C_{mic}/DOC indicate greater efficiency of utilization of organic C (Anderson 2003).

DNA abundance analysis

DNA was extracted and purified from three replicates per sample (0.25 g field moist soil) using a PowerSoil DNA kit (MO Bio Laboratories) per the manufacturer's instructions. After extraction,

Table 1. Mean levels of ammonium (NH_4-N), nitrate (NO_3-N), phosphate (PO_4), and total mineral nitrogen (TMN) in soil samples from secondary and *Pentaclethra*-dominant forests within the Maquenque National Wildlife Refuge in the Northern Zone of Costa Rica.

Forest Type	NH_4-N (μg cm^{-3} dry soil)	NO_3-N (μg cm^{-3} dry soil)	PO_4^{3-} (μg cm^{-3} dry soil)	TMN (μg cm^{-3} dry soil)
Secondary Forest	5.54 ± 1.35	1.72 ± 0.42	2.6 ± 0.45	6.39 ± 0.21
Pentaclethra Forest	4.95 ± 1.17	1.94 ± 0.37	2.93 ± 0.25	6.27 ± 0.13
Hedge's *d*	0.41	0.47	0.81	0.28
P	0.47	0.42	0.24	0.65
PD	10.60	12.80	117.00	1.90

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

the replicates were pooled and the purity of the DNA samples was checked by assuring that all A230 values were < 0.1. Soil DNA concentrations (ng DNA μL^{-1}) were determined by 1.5 % agarose gel electrophoresis and Gene Tools software (Synoptics Limited, Frederick, MD).

Endpoint PCR (EPCR) was performed on the purified DNA samples to determine presence of the target genes in each sample using AmpliTaq Gold (Applied Biosystems, Foster City, CA) complete Master Mix with the suppliers recommended PCR temperature schedule, and the *Rhizobium* 16s rRNA primer set (Singh *et al.* 2006) 63f (5'-AGGCC TAACACATGCAAGTC-3') and Rhiz-1244r (5'-CTCGCTGCCCACTGTCAC-3'), the methanotroph 16s rRNA primer set (Chen *et al.* 2007) Type IIF (5'-GGGAMGA TAATGACGGTACCWGGA-3') and Type IIR (5'-GTCAARAGCTGGTA AGGTTC-3')-both at an annealing temperature of 60 °C-and the Archaea 16s rRNA primer set (Kemnitz *et al.* 2005) A364Af (5'-CGGGGYGCASC AGGCGCGAA-3') and A934b (5'-GTGCTCCCCCGCCAATTCCT-3') at a 66 °C annealing temperature.

The relative abundance (RA) of each target gene was estimated using a quantitative PCR assay using the same primers and temperature schedule as above and a MJ Research Opticon one thermal cycler. The abundance of the target gene 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 calculated as the ratio of the abundance of that gene in a sample to the total abundance of all genes combined in that same sample.

Microbial diversity analysis

Restriction fragment length polymorphism (RFLP) analysis was conducted on each EPCR-positive sample to assess the relative diversity of the microbial communities. The EPCR products were purified and concentrated using the Wizard SV Gel and PCR Clean-Up System (Promega Corporation) and digested using restriction enzymes from Fermentas Enzymes (Fermentas Inc.), following the manufacturers' protocols. Methanotroph EPCR product DNA was digested using an equimolar combination of Taq1, Hae3 and Rsa1; the *Rhizobium* EPCR product DNA was digested using equimolar amounts of HhaI, MspI, and RsaI; and the Archaea EPCR product DNA was digested with TaqI. The digested DNA was analyzed by electrophoresis on a 2 % agarose gel followed by GeneTools (Synoptics Limited) to determine the RFLP-based richness (S), diversity (H′), and evenness (E) indices for the different plots and habitat types (Shannon & Weaver 1963).

Statistical analysis

Statistical analyses were performed using the statistical data-visualization system Mondrian (Augsburg University) and RT4Win to determine if there were meaningful differences in the mean values of the parameters measured from the different habitats. The percent difference (PD), the t-Test *P* values, and Hedge's *d* effect size statistic (> 0.7 is considered a large effect size difference) were used as indicators of biologically meaningful differences between mean values of parameters measured, as recommended for analysis of small sample sizes by Di Stefano *et al.* (2005). Pearson's Correlation methods were used to examine the data for relationships between factors. Any two factors resulting from this analysis with *r* values

Table 2. Soil dissolved organic carbon (DOC), microbial biomass (C_{mic}), carbon use efficiency metrics (C_{mic}/DOC and qCO_2), and respiration analyses of soil samples from the secondary and *Pentaclethra*-dominant forests within the Maquenque National Wildlife Refuge in the Northern Zone of Costa Rica.

Forest Type	DOC (mg cm^{-3} dry soil)	C_{mic} (µg cm^{-3} dry soil)	Percent C_{mic}/DOC	qCO_2	Respiration (mg cm^{-3} soil h^{-1})
Secondary Forest	706.5 ± 4.38	166.90 ± 4.08	23.74± 6.36	0.018 ± 0.003	2.97 ± 0.33
Pentaclethra Forest	726.74 ± 123.37	63.43 ± 39.17	8.90 ± 5.05	0.068 ± 0.56	2.85 ± 0.96
Hedge's *d*	0.19	2.25	2.25	1.1	0.14
P	0.8	0.014	0.029	0.057	0.82
PD (%)	10.60	12.80	117.00	1.90	4.00

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

Table 3. Percent relative abundances (RA) of *Rhizobium*, Methanotroph, and Archaea population rDNA in secondary forest and *Pentaclethra*-dominant forest soils within the Maquenque National Wildlife Refuge in the Northern Zone of Costa Rica.

Forest Type	RA *Rhizobium*	RA Methanotrophs	RA Archaea
Secondary Forest Soil	23.7 ± 48.2	4.1 ± 6.4	67.7 ± 7.71
Pentaclethra Forest Soil	59.4 ± 25.9	10.9 ± 17.3	15.4 ± 7.1
PD (%)	150.60	68.80	81.5
P value	0.20	0.64	0.0001
Hedge's *d* value	0.86	0.30	6.13

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

Table 4. Critical correlation results (defined as having *r* values $\geq$ 0.443 or $\leq$ -0.443, with *P* values $\leq$ 0.2) from comparisons between parameters measured in the soil in secondary forest and *Pentaclethra*-dominant forest soils within the Maquenque National Wildlife Refuge in the Northern Zone of Costa Rica. Pearson's Correlations were made between phosphate (PO_4), total mineral nitrogen (TMN), microbial biomass (C_{mic}), carbon use efficiency metrics (C_{mic}/DOC and qCO_2), dissolved organic carbon (DOC), and the relative abundances (RA) of *Rhizobium*, Methanotroph, and Archaea. Only the *r* values and associated *P* values (in parentheses) of the critical correlations are presented (NC = not critical).

	C_{mic}	C_{mic}/DOC	qCO_2	RA *Rhizobium*	RA Methanotroph	RA Archaea
PO_4	-0.616 (0.104)	-0.541 (0.166)	NC	NC	NC	NC
TMN	NC	NC	NC	0.613 (0.106)	NC	NC
DOC	NC	NC	NC	NC	0.696 (0.055)	NC
C_{mic}	NC	0.991 (0.000)	-0.781 (0.022)	NC	NC	0.593 (0.122)
C_{mic}/DOC	NC	NC	NC	-0.778 (0.023)	NC	0.647 (0.083)
qCO_2	NC	NC	NC	NC	0.603 (0.114)	NC

Table 5. RFLP-based Shannon-Weaver diversity indices for Archaea, *Rhizobium* and methanotroph, DNA from secondary forest soils and *Pentaclethra*-dominant 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*).

Forest Type	Archaea *S*	Archaea *H´*	Archaea *E*
Pentaclethra	9.0 ± 0.82	1.87 ± 0.20	0.81 ± 0.08
Secondary	9.5 ± 1.29	2.02 ± 0.11	0.84 ± 0.03
PD(%)	10.00	8.00	3.70
P value	0.8	0.25	0.43
Hedge's *d*	0.24	0.74	0.51

Forest Type	Rhizobium *S*	Rhizobium *H´*	Rhizobium *E*
Pentaclethra	5.0 ± 0.00	1.25 ± 0.05	0.90 ± 0.04
Secondary	5.0 ± 0.00	1.12 ± 0.09	0.69 ± 0.06
PD (%)	0	11.60	30.40
P value	NA	0.04	0.001
Hedge's *d*	NA	1.55	3.74

Forest Type	Metha-notroph *S*	Metha-notroph *H´*	Metha-notroph *E*
Pentaclethra	1.50 ± 1.73	0.13 ± 0.16	0.12 ± 0.15
Secondary	3.50 ± 1.73	0.85 ± 0.57	0.58 ± 0.39
PD (%)	50.00	75.00	59.70
P value	0.05	0.02	0.1
Hedge's *d*	1.52	1.87	1.18

≥ 0.443 or ≤ -0.443, with P values ≤ 0.2 were consi-dered critical correlations based on standard statistical tables for critical values of r.

Results

Nutrient analyses yielded no notable differences in levels of NH_4-N, NO_3-N, total mineral nitrogen (TMN), dissolved organic C (DOC), and respiration, but somewhat greater PO_4 levels were found in the *Pentaclethra*-dominant forest soils (Tables 1 & 2). The microbial biomass and C_{mic}/DOC were greater and the qCO_2 was less in the secondary forest soils than in the *Pentaclethra*-dominant soils (Table 2).

Major differences in the microbial community structure were identified using DNA abundance and microbial diversity analyses. The relative abundance (RA) of Archaea was greater in the soil from the secondary forest than the *Pentaclethra*-dominant forest, but the reverse was the case for the RA of *Rhizobium* in these soils (Table 3). The RA of methanotrophs was about the same in both soil types. There were strong negative correlations (Table 4) between levels of soil PO_4 and both C_{mic} (r = -0.616, P = 0.104) and C_{mic}/DOC (r = -0.541, P = 0.166), and between qCO_2 and both C_{mic} (r = -0.781, P = 0.022) and C_{mic}/DOC (r = -0.778, P = 0.023). There were strong positive correlations (Table 4) between the RA of Archaea and both C_{mic} (r = 0.593, P = 0.122) and C_{mic}/DOC (r = 0.647, P = 0.083), between the RA of methanotrophs and the DOC (r = 0.696, P = 0.055) and qCO_2 (r = 0.603, P = 0.114), and between the RA of *Rhizobium* and TMN (r = 0.613, P = 0.106). The diversity of *Rhizobium* and Archaea was greater in the *Pentaclethra*-dominant forest soils, that of methanotrophs was greater in the secondary soils, and general microbial diversity was greatest in the secondary forest soil (Table 5).

Discussion

This study was the first of its kind to examine the soil community structure of the *Rhizobium*, Archaea, and Methanotrophs and associated C and N cycle components within two lowland forest types in Costa Rica. The levels of mineral N and DOC were about the same for the secondary and the *Pentaclethra*-dominant forest types. However, the secondary forest soils had greater microbial biomass, and were more efficient at utilizing organic C, and had a greater abundance of Archaea DNA than the *Pentaclethra*-dominated forest soils. By contrast, *Pentalethra* soils had more *Rhizobium* DNA than the secondary forest soils. This difference in microbial group abundance, biomass and C-use efficiency are ecologically important, since these metrics have been associated with more complex soils and greater amounts of organic C becoming available to the microbiota (Anderson 2003; Anderson & Domsch 1989; Brookes 1995; He *et al*. 2003; Moscatelli *et al*. 2005). This suggests that the secondary forests have a more complex and efficient soil community structure, consistent with the greater complexity of the above-ground biomass found there.

Larger Archaea populations in soils have been associated with more complex organic C composition and increased biomass in soil, especially the upper layers (Chelius & Triplett

2001; Hallam *et al.* 2006; Rutz & Kieft 2004; Schleper *et al.* 2005; Simon *et al.* 2000, 2005; Sliwinski & Goodman 2004; Treusch *et al.* 2005; Wessén *et al.* 2010). The greater RA of Archaea and the strong correlations between microbial biomass and C use efficiency suggest that this microbial group is important in the C cycle of these soils. It is also possible that the Archaea in these forests are involved in AO activity, as this has capacity has been demonstrated in Archaea within forest soil ecosystems (Hallam *et al.* 2006; Schleper *et al.* 2005; Treusch *et al.* 2005).

It is of interest to note that greater Archaea abundance was associated with a lower abundance of *Rhizobium* in the secondary forest, even though there was no difference in mineral N between the two soils. It may be that these results reflect the presence of the nifH genes for N-fixation in some of the Archaea. Quaiser *et al.* (2002) identified a gene cluster with high level of similarity in structure and sequence to the fixABCX operons associated with N-fixation in many symbiotic N-fixing soil bacteria, although it is not known at this point if this operon is used for N-fixation in these Archaea. More recently, Pernthaler *et al.* (2008) provided evidence that the Archaea from methane seeps participate in N-fixation, and Miyazaki *et al.* (2009) identified that Archaeal microbial communities in the Eel River basin have the nifH gene and could be involved in N-fixation. Thus, more evidence is coming forward which infers that some members of Archaea may be involved in this N cycle activity, although the role of the terrestrial Crenarcheaota is not yet clear. Nonetheless, there appear to be interactions and community structural changes occurring among the *Rhizobium* and Archaeal populations within the soil from these two forest types which can consequently affect C and N cycling.

Regardless of their specific roles, which can only be speculated at this point, Archaea appear to be more dominant than *Rhizobium* in the secondary forests, while the opposite is the case in the *Pentaclethra*-dominant forest soils. This is the first indication that a soil Archaeal community may be playing a significant role in both N and C cycle dynamics in tropical soils. More research is needed to confirm these propositions.

Methanotrophs can enhance forest soil organic C content (Bastviken *et al.* 2003; Bull *et al.* 2000; Hanson & Hanson 1996; Mancinelli 1995; Murase & Frenzel 2007; Whalen *et al.* 1990), be involved in N-fixation in forest soils (Auman *et al.* 2001; Chu & Alvarez-Cohen 1999), and interact with methanogenic Archaea (e.g. Hanson & Hanson 1996). Although there was little difference in the abundance of this community between the secondary and *Pentaclethra*-dominant forest soils, the methanotrophic population in the secondary forest soils was much more diverse, and there was as strong positive correlation between methanotroph RA and DOC. This suggests that in the secondary forests, where there is less N-fixation due to *Pentaclethra,* there may be more methanotrophic species performing this function as well as playing an important role in organic C composition.

Conclusions

Pentaclethra macroloba is a shade-tolerant species that tends to colonize small gaps or along the edges of smaller gaps in neotropical forests (Hartshorn 1980; Oberbauer & Strain 1985). This tree is, thus, capable of establishing populations during early succession in tropical forests (Palomaki *et al.* 2006). In this study, we demonstrated that within two forest types in the MNWLR, there are clear differences in three microbial community components critical to the C and N cycles of these two habitats, differences that are consistent with those in the above-ground vegetation. Specifically, the secondary forest soils had a greater total microbial biomass and efficiency of C utilization, greater relative abundance of methanotrophs and Archaea 16S rDNA, and greater overall microbial diversity, whereas the relative abundance of *Rhizobium* was greater in soils from forests that were dominated by *Pentaclethra macroloba*. The data suggest that rhizobia, methanotrophs and Archaea are involved in a complex interplay that affects the C and N cycle dynamics.

Acknowledgements

The authors thank Caitlin Looby for her assistance in obtaining samples and data, and the rest of the REU students for their support. We would also like to thank Kurt Schmack and the staff members at the Laguna del Lagarto Lodge in Boca Tapada, Alajuela, Costa Rica. This study was supported by a grant from the National Science Foundation (DBI-0452328) and was conducted under the Costa Rican Government Permit #063-2008-SINAC.

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(Received on 20.05.2011 and accepted after revisions, on 15.07.2011)