Biogeochemistry (2008) 89:209–220
DOI 10.1007/s10533-008-9214-7

ORIGINAL PAPER

Soil aggregates in a tropical deciduous forest: effects on C and N dynamics, and microbial communities as determined by t-RFLPs

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Received: 27 August 2007 / Accepted: 20 May 2008 / Published online: 7 June 2008

Abstract The aim of this study was to analyze C and N dynamics, as well as, soil bacterial community structure within soil micro- and macro-aggregates in a tropical deciduous forest in México. We measured, for three landscape positions and three seasons of the year: total, microbial and available forms of C and N; potential C and N mineralization; and soil bacterial communities by using t-RFLPs. The highest total C concentrations were found in the north-slopes and in the dry season (DS) samples. In general, micro-aggregates had higher concentrations than macro-aggregates of available C and N forms, and microbial C. Similarly, micro-aggregates had the highest potential C mineralization and net N mineralization. We detected 149 different OTUs (operational taxonomic units) from which 50% was shared by the two aggregate size fractions, 25% was exclusive to micro-aggregates and the 25% left was found only in macro-aggregates. Top-hills were richer in OTUs than north and south-slopes. The Unweighted Pair Group Method with Arithmetic mean (UPGMA) analysis indicated clear differences in community composition between the two aggregate size-fractions in relation to the presence of OTUs. These results suggest that the main difference between micro- and macro-aggregates is due to the community structure within each soil fraction and this difference could affect soil nutrients dynamics.

Keywords Macro-aggregates · Micro-aggregates · Mexico · Soil bacteria · t-RFLPs

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Introduction

Soil organic matter distribution and turnover rates are highly related to soil organisms such as fungi, bacteria, and earthworms and also to soil aggregate dynamics (Elliott 1986; Six et al. 2004). Aggregates play an important role in soil structure stability, soil organic carbon protection, nutrient availability, as well as, differential microbial activity (Beare et al. 1994; Six et al. 2000a, b; Denef et al. 2001; García-Oliva et al. 2004).

Tisdall and Oades (1982) suggested a soil aggregation model which states that primary mineral

particles are bound together to form micro-aggregates. Sequentially, micro-aggregates form macroaggregates by binding agents of different nature and stability: (1) transient, with resident time of weeks, and formed mainly by microbial and plant derived polysaccharides and; (2) temporary, which may persist from months to a few years, and consist of roots, fungal hyphae, bacterial cells and algae (Six et al. 2000b). Micro-aggregates are protected within stable macro-aggregates and when the latter break micro-aggregates are released along with a liberation of labile soil organic mater, enhancing microbial activity (Beare et al. 1994; Gupta and Germida 1988). Some studies reported that microbial activity is favored within macro-aggregates mainly in sandy soils, because they reduce the impact of variation in soil chemical and physical environmental conditions (Hassink et al. 1993; García-Oliva et al. 2004). According to Elliott (1986) organic matter contained in macro-aggregates is more labile than the organic matter restricted to micro-aggregates. Other authors have found that soil organic matter associated to macro-aggregates is less decomposed and the organic matter linked to micro-aggregates is highly processed by microbial activity (Elliott 1986; Gupta and Germida 1988; Oades and Waters 1991; Tisdall and Oades 1979).

Aggregate soil fraction and their environmental heterogeneity influence microorganisms' distribution as well as the different processes they carry out (Mendes and Bottomley 1998; Mendes et al. 1999; Grundman and Dubouzie 2000; Ettema and Wardle 2002). Recently, different studies have shown the non-random distribution of bacteria in soils (Norris et al. 2002; Nunan et al. 2003; Grundmand 2004; Noguez et al. 2005), and also the microbial biomass variation and differential colonization among different sizes of aggregates (Van Gestel et al. 1996; Mendes et al. 1999; Ranjard and Richaume 2001; Schutter and Dick 2002). As an example of this are *Actinobacteria* species which dominate within inner micro-aggregates of undisturbed soils (Mummey and Stahl 2004).

Soil microbial communities are key intermediates in different biogeochemical processes. In turn, soil organic matter decomposition and soil respiration rates can be strongly affected by microbial community structure (Padmanabhan et al. 2003; Cleveland et al. 2007). We know that soils with greater amounts of basal resources can sustain microbial communities with more diverse functional activities (Degens et al. 2000). However, we do not know the majority of the microbial communities present in the soil and their roles in soil processes and how their activities are affected at local scale by soil aggregation. There is just little information about the role of community structure on different soil processes (Balser and Firestone 2005) and more specifically within the aggregate soil fractions. The purpose of this work was to characterize soil bacterial community structure within soil macro- and micro-aggregates and the possible role of these communities in C and N dynamics in a tropical deciduous forest in western Mexico.

Methods

Study site

The study was performed in the Chamela-Cuixmala Biosphere Reserve on the Pacific coast of Mexico (19°30′ N and 105°01′ W), within a small watershed system that has been extensively studied as part of a research program on ecosystem functioning (Maass et al. 2002). Predominant landscape forms are low hills with steep slopes (<20°) and the dominant vegetation is tropical deciduous forest (TDF; Bullock and Solís-Magallanes 1990). The forest consists mainly of deciduous trees (6–10 m in height) most of which are leafless for several months each year (Lott et al. 1987). There are 107 plant families within the Biological Station, represented by 758 species. The most abundant trees belong to the families Leguminosae, Apocynaceae, Cochlospermacea, Euphorbiaceae, Boraginaceae and Burseraceae, being the Leguminoseae the most important family, representing 15% of the species (Segura et al. 2003). Mean annual temperature is 24.6°C and mean annual precipitation 788 mm (García-Oliva et al. 2002). Soils are Eutric Regosols (Cottler et al. 2002) poorly developed, with a pH of 6.9 (García-Oliva and Maass 1998). Around 30% of soil organic matter (García-Oliva and Maass 1998) and about 76% of the fine root productivity occur in the first five centimeters (Castellanos et al. 2001). The proportion of macro-aggregates found in the forest soils constitutes about 80% of the total soil and they play an important role in the short and long-term C

storage (Garcia-Oliva et al. 1999; Garcia-Oliva and Tapia 2001).

Soil sampling

We used a design with three factors: (1) three landscape positions (south-facing mid-slopes, north-facing mid-slopes and top-hills); (2) two soil aggregate size fractions (micro-aggregates <250 μm and macro-aggregates >250 μm) and; (3) three sampling dates: dry season (*DS*; May), early rainy season (*ERS*; June) and rainy season (*RS*; September). This design was employed in order to test if the functional role of macro-aggregates and micro-aggregates was independent of the different contents of soil organic matter and soil humidity present in the three landscape positions chosen. Sampling sites with different slope aspect, within the watershed, present different solar radiation indexes (SRI; 3651 MJ m^{-2} $year^{-1}$, north aspect; 4475 MJ m^{-2} $year^{-1}$, south aspect; 4273 MJ m^{-2} $year^{-1}$; Galicia et al. 1999) and different soil nutrients and water content (Galicia et al. 1999). Within the experimental watershed system hill-slopes and top-hills units were identified according to their longitude, aspect, as well as edaphic and morphological similarities (López-Blanco et al. 1999). In November 2001 seven plots, 150 m^2 in area, were established for each of the three landscape positions (south-facing mid-slopes, north-facing mid-slopes and top-hills); and the sampling was conducted during 2002. In order to account for within site variation, we took randomly (in each plot and in each sampling date) 15 undisturbed topsoil cores (0–5 cm), these were mixed thoroughly to obtain one composite sample per plot. From these composite samples, the DNA was extracted immediately. Then, the samples were stored in black plastic bags and kept in refrigeration at 4°C, until biogeochemical parameters were determined.

Soil biogeochemical analyses

Prior to the biogeochemical analyses, soil samples were sieved (at field moisture) in two aggregate size fractions, micro-aggregates (<250 μm) and macro-aggregates (>250 μm), small pebbles and sand material were excluded manually. Before total forms analyses, samples were dried and then ground with mortar and pestle. Total C (Ct) was determined by using an automated CO_2 analyzer (UIC, mod. CM5012, IL USA). Total N (NT) was analyzed by a macro-Kjeldahl method (Technicon Industrial System 1977) and colorimetric readings were done using an auto-analyzer (Bran + Luebbe 3 Auto Analyzer, Germany).

Microbial C (Cm) and N (Nm) were determined in field-moist samples, according to the $CHCl_3$ fumigation-extraction method (Vance et al. 1987). Fumigated and non-fumigated samples were incubated during 24 h at 25°C at constant moisture content. Samples were fumigated for 24 h with ethanol-free chloroform at 24°C in dark conditions. Subsequently, chloroform was removed by evacuation. Cm was extracted from both fumigated and non-fumigated samples with 0.5 M K_2SO_4, filtered using Whatman No. 42 paper and Cm measured using an automated CO_2 analyzer (UIC, mod. CM5012). The amount of Cm was calculated as the difference between non-fumigated and fumigated samples divided by the efficiency value Kc of 0.45 (Joergensen 1996). Nm was extracted in a similar way as Cm; it was first filtered through a Whatman No. 1 paper, and then the collected solution was acid digested and determined as total N by a macro-Kjeldahl method (Brooks et al. 1985). Nm was calculated similarly to microbial C, but divided by a Kn value of 0.54 (Brookes et al. 1985). The values of microbial C and N were divided by their corresponding weight of dry soil. The amount of K_2SO_4-extractable C obtained from non-fumigated soil was used as a measurement of soil labile C (C_{labile}). Using fresh samples, NH_4^+ and NO_3^- were extracted from 5 g soil sub-samples by shaking for 30 min with 50 ml of 2 M KCl. The extracts were filtered and nitrate and ammonium were determined colorimetrically using a Braun + Luebbe 3 autoanalyzer.

To estimate potential C mineralization aerobic incubations were performed in the laboratory: 100 g of fresh soil samples were placed in a PVC (polyvinyl-chloride) core (3.5 cm in diameter) with a 0.17 mm mesh at the bottom. Samples were incubated in 1 l jars for 15 days at 25°C at field capacity. The evolved CO_2–C was collected in traps containing 10 ml of 1 M NaOH solution. CO_2–C concentration in the traps was determined by adding 5 ml 1.5 M $BaCl_2$, and titrated with 0.5 M HCl (Coleman et al. 1978). The jars were regularly aerated, the CO_2–C traps changed, and the soil moisture was periodically adjusted to reflect field capacity by adding deionized

water. The CO_2–C values were divided by their corresponding weight of dry soil. After incubation, soil samples were analyzed for NH_4^+ and NO_3^- with the methods described previously. Net nitrification was calculated as the difference between pre- and post-incubated concentrations of NO_3^- and net nitrogen mineralization was calculated as post-incubated ammonium—plus nitrate–nitrogen minus the sum of pre-incubated ammonium—and nitrate–nitrogen. Final values were adjusted according to moisture content and reported in terms of the mass of oven-dried soil.

Statistical analyses

All statistical analyses were performed with Statistica 6 software (StatSoft 2000). The effect of the three factors considered (position, aggregate size and time) on nutrient concentration was analyzed by a repeated-measured ANOVA; with two between-subject factors (landscape position and aggregate size) and one within-factor treated as repeated measured (sampling date). A Greenhouse-Geisser correction for time factor was used when data did not meet the circularity assumption of the repeated measured analysis (von Ende 1993).

We had three levels for landscape position (top-hills, south-facing mid-slopes and north-facing mid-slopes), with seven replicates for each position; two levels for aggregate size (>250 μm and <250 μm) and three levels for the sampling date factor (*DS*, *ERS* and, *RS*). Data were log-transformed to meet MANOVA assumption when required. When the MANOVA indicated significant factor effects, Tukey's HSD multiple comparison test was used.

DNA extraction and TRFLPs

Another fraction of the soil samples were sieved, at field moisture, into micro-aggregates (<250 μm) and macro-aggregates (>250 μm). For both aggregate fractions we extracted the genomic DNA the same day of sampling using the Ultra Clean Soil DNA Kit (Mo Bio Lab., Inc.), and stored the products at −20° C. From each sample we amplified by PCR the 16S rRNA genes, using fluorescently labeled domain-specific primers (Forward 515 VIC 5′-GCGGATCCTCTAGA CTGCAGTGCCAGCAGCCGCGGTAA-3′; Reverse 1492 6FAM 5′-GGCTCGAGCGGCCGCCCGGGT TACCTTGTTACGA CTT-3′, Applied Biosystems). Three independent PCR were performed for each sample. Each PCR reaction containing 1 × PCR Buffer, 1.65 mM $MgCl_2$, 0.2 mM dNTP mixture, 0.6 μM of each primer, 1 unit Taq polymerase and 5% BSA. All reactions were carried out in a MJ research thermocycler with the following program: 94°C × 4 min; 35 cycles 92°C × 1.5 min, 50°C 1.5 min, 72°C × 2 min; 72°C × 10 min. PCR products were combined and purified from a 2% agarose gel (Gel extraction kit Qiagen, Inc.). The amplicons were restricted using *AluI* and *RsaII*, in a 20 μl reaction during 3 hours, one reaction for each enzyme (Promega). Each reaction contained 10 units of enzyme (*AluI* or *RsaII*) enzyme and 50 ng of the PCR product, and was incubated at 37°C for 3 h then at 65°C for 30 min. The sizes and abundances of fluorescently labeled terminal restriction fragments (t-RFs) were determined using an ABI 3100 PRISM DNA Genetic Analyzer (Applied Biosystems).

Bacterial community analysis

Each t-RF was considered to be an operational taxonomic unit (OTU) and only those OTUs with heights ≥50 fluorescent units were used for the analyses (Blackwood et al. 2003). In order to compare microbial communities between samples and sites we constructed a present/absence matrix using the OTUs information. We estimated the co-ocurrence probabilities of OTUs between the two soil aggregate fractions and we constructed rarefaction curves in order to compare richness between aggregates and among the different positions considered, using the EcoSim Program (Gotelli and Entsminger 2001). We also calculated the Shannon diversity index for the two sizes of aggregates. In order to evaluate similarity and to assesss microbial distribution among the different aggregate soil samples we performed an Unweighted Pair Group Method with Arithmetic mean analysis based on the presence/absence of OTUs (UPGMA, Sneath and Sokal 1973) using PAUP* 4.0 (Swofford 2000). We carried out a stepwise regression for each soil aggregate fraction, and the September data, to determine if there was a relationship between the number of OTUs found and microbial and available forms of C and N (labile C, microbial C, microbial N, NH_4^+ and NO_3^-).

Results

Soil biogeochemical parameters

Total forms

The highest total C concentrations were found in samples from the north-facing mid-slopes, independently of the aggregate size-fraction and sampling date (Tables 1 and 2). Nevertheless, micro-aggregates had higher C values than macro-aggregates (43.6 and 28.8 mg C g^{-1}, respectively). In general, total C concentration diminished from the DS to the RS (Tables 1 and 2). In the DS, micro-aggregates had higher total N concentration than macro-aggregates within the three landscape positions, although these differences were not significant in the two rainy season dates (ERS and RS; Tables 1 and 2). We observed that total nitrogen tendencies throughout seasons were different. Whereas within micro-aggregates N decreased with sampling date, within macro-aggregates nitrogen concentrations showed no changes (Table 2). As for the total C/N ratio the tendency was to increase with sampling date in micro-aggregates, but there was no season effect in macro-aggregates (Table 1).

Available forms

Labile C concentrations were higher in micro-aggregates than in macro-aggregates for both the DS and the ERS samples; however, samples from the RS were not significantly different between micro- and macro-aggregates (Tables 1 and 2); moreover, in both aggregate size-fractions the RS samples had lower significant labile C values than the previous seasons (Table 2). In the case of ammonium, micro-aggregates had significantly higher concentrations than macro-aggregates (19.4 μg NH_4 g^{-1} and 10.3 μg NH_4 g^{-1}, respectively) independently of the sampling date or the landscape position. At the same time, samples from the DS and the ERS had similar values (12 μg NH_4 g^{-1} and 13 μg NH_4 g^{-1}, respectively); but these values were smaller than the values found for the RS samples (19.1 μg NH_4 g^{-1}; Table 1). Size effect for nitrates was significant only for the RS samples, in which micro-aggregates had higher NO_3^- concentration (112 μg NO_3^- g^{-1}) than macro-aggregates (89 μg NO_3^- g^{-1}). Nitrate values for the RS samples, for both size fractions, were approximately five times higher (100 μg NO_3^- g^{-1}) than the values found in the previous sampling dates (21 and 17 μg NO_3^- g^{-1} for the DS and the ERS,

Table 1 *F*-ratios and significant levels of the repeated measures ANOVA for soils in a tropical deciduous forest at Chamela, Mexico

Parameters	Source of variation						
	Between subject			Within subjects			
	Position (P)	Size (S)	P × S	Date (D)	D × P	D × S	D × P × S
Total carbon	8.4***	38.8***	1.3^{ns}	4.6**	1.9^{ns}	2.3^{ns}	0.1^{ns}
Total nitrogen	8.8***	130.2***	0.2^{ns}	49.8***	0.6^{ns}	71.5***	2.6*
C:N	0.2^{ns}	14.5***	0.5^{ns}	7.5***	0.6^{ns}	26.8***	1.4^{ns}
C-lab[a]	2.2^{ns}	31.7***	0.1^{ns}	78.1***	1.6^{ns}	20.2***	1.8^{ns}
Ammonium	1.7^{ns}	27.3***	0.7^{ns}	9.9***	2.2^{ns}	1.5^{ns}	1.7^{ns}
Nitrate	59.3***	14.2***	0.6^{ns}	535.6***	32.8***	12.0***	0.9^{ns}
Microbial C	2.1^{ns}	20.2***	0.3^{ns}	167.2***	0.2^{ns}	20.6***	2.2^{ns}
Microbial N	1.4^{ns}	29.4***	6.1**	90.8***	3.7**	17.1***	6.2***
Microbial C:N	2.1^{ns}	0.4^{ns}	0.8^{ns}	0.9^{ns}	0.5^{ns}	1.4^{ns}	0.2^{ns}
CO_2–C[b]	5.7**	43.9***	7.4**	414.0***	4.5**	3.4*	4.8**
N mineralization	11.9***	24.4***	4.3*	12.2***	0.7^{ns}	0.8^{ns}	0.2^{ns}
Net nitrification	9.8***	4.2*	5.5**	4.1***	0.8^{ns}	2.6^{ns}	0.4^{ns}

Note: ns = not significant

[a] Labile carbon

[b] Potential C mineralization rate

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 2 Seasonal means (± standard error) of total nutrient concentrations and C:N ratio associated with two soil aggregate size-fractions at three landscape position in a tropical deciduous forest in Chamela, Mexico

	Macro-aggregates			Micro-aggregates		
	Top-hill	South-slope	North-slope	Top-hill	South-slope	North-slope
	Total C (mg g^{-1})					
DS	28.4(3)	26.0(2)	34.2(3)	43.9(3)	44.5(3)	58.6(6)
ERS	26.6(1)	24.2(3)	32.9(3)	39.7(2)	37.1(2)	51.0(5)
RS	25.7(2)	22.6(2)	34.3(5)	34.1(2)	36.0(4)	51.6(6)
	Total N (mg g^{-1})					
DS	2.3(0.2)^{a*}	2.1(0.1)^{a*}	2.9(0.4)^{a*}	6.7(0.8)^{a}	6.9(0.5)^{a}	7.9(0.3)^{a}
ERS	2.4(0.1)^{a}	2.2(0.1)^{a}	2.9(0.3)^{a}	4.4(0.3)^{b}	4.2(0.3)^{b}	6.2(0.3)^{b}
RS	2.4(0.2)^{a}	2.3(0.2)^{a}	3.7(0.5)^{a}	3.0(0.2)^{b}	3.4(0.4)^{b}	3.5(0.4)^{b}
	C:N ratio					
DS	11.9(0.6)	12.6(0.8)	12.1(0.5)	7.3(1.2)	6.6(0.4)	7.6(1.0)
ERS	11.2(0.6)	11.2(1.3)	11.2(0.5)	9.1(0.3)	8.8(0.5)	8.2(0.7)
RS	10.8(1.0)	10.0(0.6)	9.5(0.8)	11.2(0.4)	11.7(2.3)	16.3(3.0)

DS: dry season; ERS: early rainy season; RS: rainy season. Values followed by different letter indicate that means are significantly different ($P < 0.05$) between dates within aggregate size fraction and landscape position; whereas * indicate that means are significantly different ($P < 0.05$) between aggregates size fraction within date and landscape position

respectively; Table 2), suggesting an active nitrification in the rainy season.

Microbial forms

The DS samples, in both aggregate fractions, had in average three times the microbial C values (2460 μg Cm g^{-1}) found in the ERS (620 μg Cm g^{-1})and the RS (720 μg Cm g^{-1}). However, micro-aggregate samples from the DS; had higher microbial C values (1490 μg Cm g^{-1}) than macro-aggregates (988 μg Cm g^{-1}), while both aggregate size-fractions had similar values in the ERS and the RS samples. We found no significant difference of microbial N concentration between the two size-fractions of aggregates in all but the south slope samples from the DS. Within macro-aggregates there were no significant differences regarding seasonality, with exception of the samples coming from the North-slope in which the DS samples had higher concentrations than the ERS samples. However, in the case of micro-aggregates the values found for the DS were higher than for the other two seasons (ERS and RS) for the three positions (Tables 1 and 3). Concerning microbial C/N ratio there was not significant effect of any of the three factors considered (position, aggregate size and sampling date; Table 1).

Potential-C and N-mineralization

CO_2–C production was similar in the two aggregate size-fractions with exception of the North-facing slope samples of the DS (Table 1 and Fig. 1). Soils from the DS samples had a greater CO_2–C production, which decreased significantly with the sampling date in the three landscape positions and within the two aggregate sizes fractions (Fig. 1). Net N mineralization within micro-aggregates was higher than within macro-aggregates in two landscape positions (South slope and North slope), while both size fractions presented comparable values in top-hill (Table 1 and Fig. 2a). Macro-aggregates had similar values among landscape positions, whereas within micro-aggregates the North slope samples had higher values than the other two sites (Fig 2a). The RS samples had the highest values, followed by the DS samples, the ERS samples had the lowest values (34, 15, and 7 μg N g^{-1}; respectively). Net nitrification had higher values within micro-aggregates than within macro-aggregates only in the North slope samples (Table 1 and Fig. 2b). The RS and the ERS samples had the highest values (18 and 16 μg NO_3^- g^{-1}, respectively), whereas the DS had the lowest value (4 μg NO_3^- g^{-1}).

Table 3 Seasonal means (±standard error) of available and microbial forms of C and N associated with two soil aggregate size-fractions at three landscape position in a tropical deciduous forest at Chamela, Mexico

	Macroaggregates			Microaggregates		
	Top-hill	South-slope	North-slope	Top-hill	South-slope	North-slope
	C-lab (μg g^{-1})					
DS	238(45)	221(38)	335(80)	565(116)	656(64)	809(122)
ERS	278(16)	312(40)	392(79)	476(32)	403(32)	511(81)
RS	78(8)	58(12)	162(29)	178(26)	195(13)	108(16)
	Ammonium (μg g^{-1})					
DS	6.9(3)	8.3(2)	4.8(3)	12.9(4)	14.3(2)	24.2(5)
ERS	11.3(2)	12.3(2)	8.0(1)	12.1(1)	20.7(4)	14.9(3)
RS	12.9(3)	17.0(2)	10.7(1)	26.6(7)	28.6(4)	19.0(2)
	Nitrate (μg g^{-1})					
DS	13.5(1)	28.8(6)	24.3(4)	14.2(1)	27.9(6)	16.8(1)
ERS	16.8(1)	15.7(1)	16.4(1)	15.0(1)	16.3(1)	21.7(6)
RS	65.9(5)	79.4(3)	123.1(7)	78.4(6)	103.1(7)	155.5(9)
	Microbial C (μg g^{-1})					
DS	1956(246)	1412(165)	2016(270)	3029(501)	3351(478)	3118(212)
ERS	710(54)	529(135)	560(55)	555(56)	317(59)	1044(200)
RS	520(46)	538(72)	792(123)	772(62)	613(51)	1076(165)
	Microbial N (μg g^{-1})					
DS	132(40)[a]	118(16)[a*]	170(22)[a]	242(41)[a]	419(58)[a]	207(13)[a]
ERS	54(4)[a]	46(12)[a]	48(5)[a]	55(6)[a]	37(7)[a]	69(7)[a]
RS	53(10)[a]	49(7)[a]	68(15)[a]	91(21)[a]	88(12)[a]	72(11)[a]

DS: dry season; ERS: early rainy season; RS: rainy season. Values followed by different letter indicate that means are significantly different ($P < 0.05$) between dates within aggregate size fraction and landscape position; whereas * indicate that means are significantly different ($P < 0.05$) between aggregates size fraction within date and landscape position

Bacterial communities

To analyze the differences in bacterial community composition between the two size fractions we conducted a TRFLP analysis of the genomic DNA extracted only from the soil sampled in September (RS), because the TRFLPs obtained for the other two sampling dates did not have the required quality to be analyzed. Similarly, we present only the results corresponding to the *Alu I* enzyme.

We found 149 different OTUs, 25% was exclusive to macro-aggregates, another 25% was restricted to micro-aggregates, and the remaining 50% was shared by the two size fractions. Those OTUs shared by the two fractions were also the most abundant, representing 94%, while the OTUs constrained to either macro or micro-aggregates were the least abundant, 4% for macro-aggregates and 2% for micro-aggregates. Moreover, Shannon Diversity Index values were

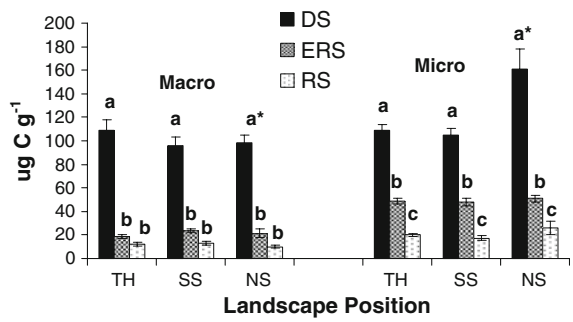

Fig. 1 Mean values of CO_2–C evolution in soil macro-aggregates and micro-aggregates at three different landscape positions (TH: top-hill, SS: south-slope and NS: north-slope), and in three different periods of the year (dry season-*DS*; onset of the rainy season-*ORS*; and rainy season-*RS*). Values followed by different letter indicate that means are significantly different ($P < 0.05$) between dates within aggregate size fraction and landscape position; whereas * indicate that means are significantly different ($P < 0.05$) between aggregates size fraction within date and landscape position

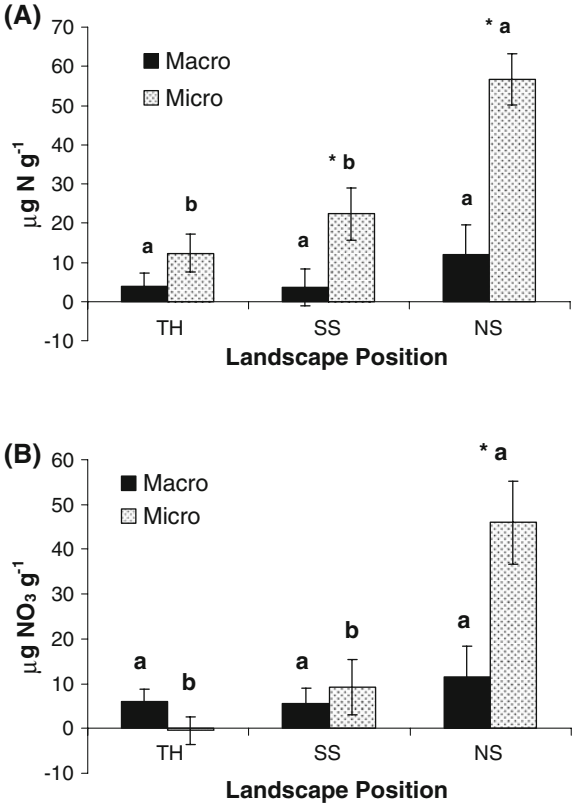

Fig. 2 (**a**) Net N Mineralization and (**b**) Net Nitrification within two aggregates size fractions and in three different landscape position (TH: top-hill, SS: south-slope and NS: north-slope).Values followed by different letter indicate that means are significantly different ($P < 0.05$) between landscapeposition within aggregate size fraction; whereas * indicate that means are significantly different ($P < 0.05$) between aggregates size fraction within landscape position

almost the same for the two soil fractions (2.8 and 2.9, respectively). The rarefaction curve shows that top-hill sites are richer than south- and north-slopes, but there is no richness difference between macro- and micro-aggregates within any of the positions considered (Fig. 3). Nevertheless, the UPGMA analysis indicated a very clear separation of the samples from the two aggregate size-fractions in relation to the presence of OTUs (Fig. 4). We found two large clades, one with OTUs inhabiting macro-aggregates (dashed line) and the other with OTUs present in micro-aggregates (solid line), with some outliers samples in both cases (Fig. 4). This suggests that both size fractions have different bacterial community composition. Finally, the number of OTUs that co-occur in the two aggregate size-fractions was lower

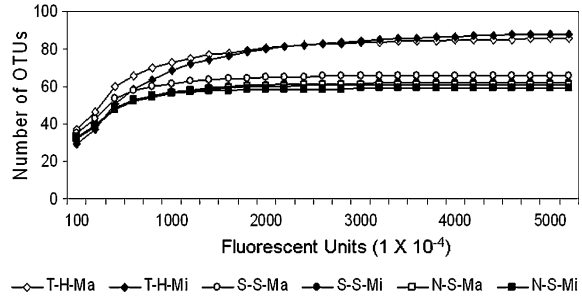

Fig. 3 Rarefaction curve base on relative abundance of OTUs measured as fluorescent units. T-H-Ma**:** macro-aggregates in the top-hill; T-H-Mi: Micro-aggregates in the top-hill; S-S-Ma: macro-aggregates in the south-facing mid-slope; S-S-Mi: micro-aggregates in the south facing mid-slopes; N-S-Ma: macro-aggregates in the north-facing mid-slopes; and N-S-Mi: micro-aggregates in the north-facing mid-slope

than expected by chance ($P < 0.05$), that is, there are OTUs that for some ecological reason can not coexist.

The stepwise regression performed indicated that for micro-aggregates the number of OTUs was positively explained only by microbial N ($r^2 = 0.20$; $P < 0.05$), while in the case of macro-aggregates it was positive explained only by NH_4^+ ($r^2 = 0.24$; $P < 0.05$).

Discussion

Soil biogeochemical parameters

Our results indicate that the influence of the position, within the landscape, is reflected only in the concentration of total C. This can be the result of the differential net primary productivity and solar radiation among the three landscape positions studied (Martínez-Yrízar and Sarukhán 1990; Martínez-Yrizar et al. 1996; Galicia et al. 1999). The solar radiation received by north slopes is lower than the radiation intercepted by the top-hills and south-facing mid-slopes; consequently, north facing mid-slopes have lower evapo-transpiration rates and higher soil humidity, resulting in a higher primary productivity (Galicia et al. 1999).

In general, we obtained significantly higher nutrient concentrations in micro-aggregates than in macro-aggregates, as observed in other studies (Ashman et al. 2003; Beare et al. 1994; García-Oliva et al. 2003). These results can be explained by the different nature of the organic matter present in each

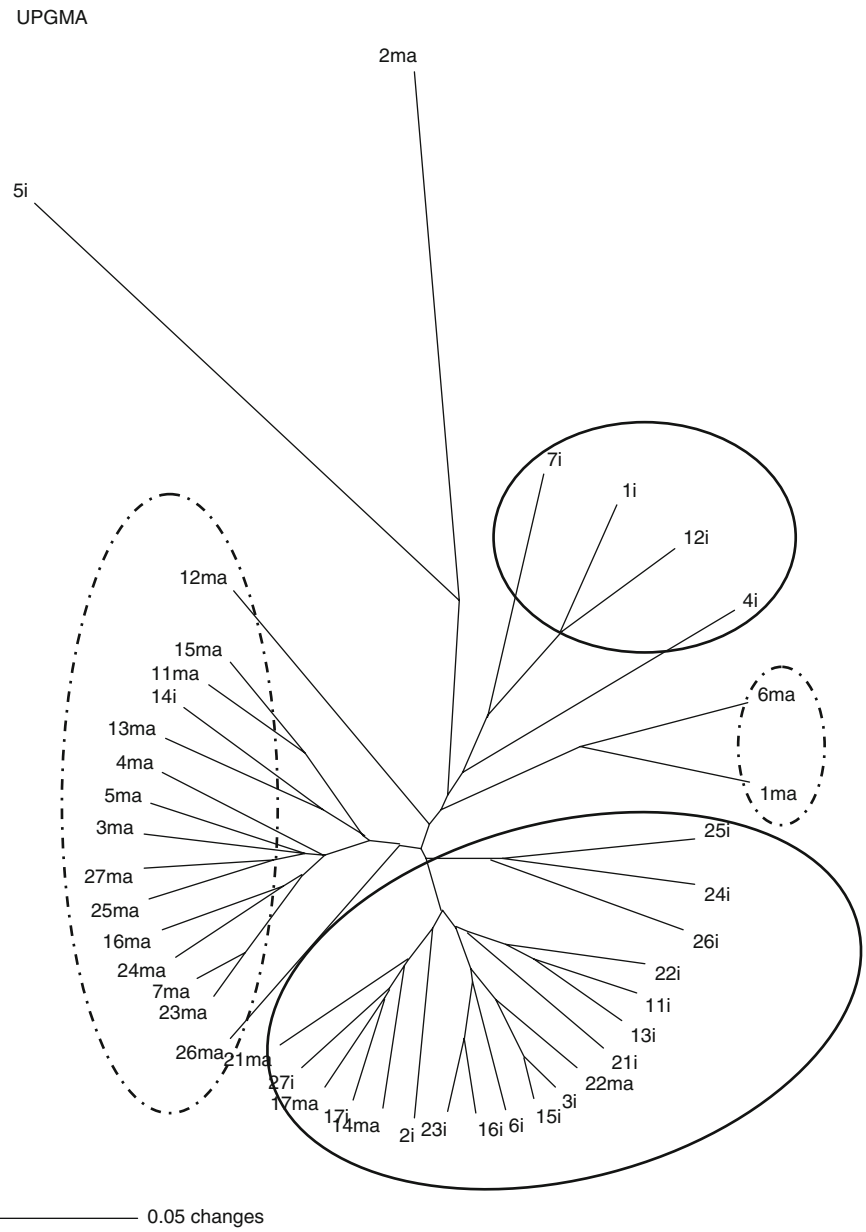

Fig. 4 UPGMA dendogram showing the degree of similarity among samples based on the presence/absence of different OTUs. Samples with ma correspond to macroaggregates and samples with i correspond to microaggregates. Solid circles grouped micro-aggregates samples and dashed circles grouped macro-aggregates samples

fraction, the differential transformation rate performed within each aggregate size; as well as the nutrient relocation from macro-aggregates to micro-aggregates, with the resulting enrichment of the small fraction (Tisdall and Oades 1982; Oades 1993; Lehmann et al. 2007).

In the RS samples, labile C plummeted in both sizes of aggregates; nonetheless, in micro-aggregates the drop was sharper than in macro-aggregates because of the higher proportion of labile and microbial C per unit of total C. It is noteworthy that for micro-aggregates the differences in labile C concentrations were significant among the three seasons studied, while for macro-aggregates there was not a significant difference between the DS and the ERS samples. The differences in labile C lost between the two fractions could be the result of: (a) the lixiviation process, (b) the extremely high activity of microbial communities within micro-aggregates and, (c) the differential organic matter availability present in the two soil fractions. The former processes give as a result a much faster consumption

rate and a lower C and N long-term storage capacity in micro-aggregates than in macro-aggregates (Oades 1984; Elliott and Coleman 1988; Oades and Waters 1991; García-Oliva et al. 2003). In our soils these results can be better explained by the higher microbial biomass, expressed as microbial C, present in micro-aggregates in the DS samples compared to macro-aggregates and, by the microbial activity. A higher microbial biomass results in a higher activity and higher labile C consumption rate; these can be supported by the higher CO_2–C evolution and net N mineralization showed by micro-aggregates during the incubation experiments.

Nitrogen redistribution and loss were very different between the two sizes of soil aggregates, total nitrogen content within macro-aggregates was very stable with time. Conversely, the loss within micro-aggregates was very sharp, and by the RS the amount of total N in this fraction was almost half that amount found during the DS. Nitrogen mineralization within micro-aggregates was higher than within macro-aggregates, with exception of the top-hill samples. These results can explain the higher concentration of inorganic nitrogen forms (NH_4^+ and NO_3^-) within microaggregates.

During the RS, NO_3^- concentration was at least three times that of NH_4^+ for both size fractions. Additionally, during this period (RS) net nitrification had the highest values independently of size fraction or landscape position. The higher nitrification in this season can be explained by the low soil C availability in these soils (Montaño et al. 2007). In this condition of low energy availability, heterotrophic microorganisms are constrained and nitrifying bacteria are favored, dominating the nitrification over microbial immobilization processes (Vitousek et al. 1982; Hart et al. 1994; Montaño et al. 2007).

Soil microbial community structure

The biogeochemical data presented in the preceding section indicate: higher nutrient concentrations in micro-aggregates than in macro-aggregates; dissimilar nature of C and N forms within each soil fraction and; differential C and N transformation rates between the two sizes of aggregates, being higher in micro-aggregates than in macro-aggregates. The differences in the biogeochemical processes carried out in the two aggregate soil fractions were also reflected in the differences in the microorganisms (OTUs) that inhabit them.

The UPGMA clearly showed that community composition, based on OTUs presence/absence matrix, is related to the aggregate size fraction. Moreover, the co-ocurrence analysis showed that many organisms do not co-exist, no matter of the landscape position in which they are located. This micro-scale approach contributes to the evidence found by other authors about the non-random and non-cosmopolitan distribution of microorganisms (Horner-Devine et al. 2004; Noguez et al. 2005).

Even though we do not know which soil organisms are carrying out which soil processes, we found that the most abundant microorganisms are in both aggregate size fractions, while those microorganisms exclusively found in macro- or micro-aggregates are the least abundant, and probably they could affect soil N transformation. This hypothesis is based on the fact that the number of OTUS correlated with different N forms in each aggregate size fraction. We can conclude that the main difference between micro- and macro-aggregates is due to the community structure within each soil fraction and this difference could affect soil nutrients dynamics. Changes in microbial composition without changes in species richness have been reported recently in tropical ecosystems (Cleveland et al. 2007). However, studies of functional species determination is needed for test this hypothesis.

Acknowledgements We thank to the anonymous reviewers for their valuable comments to this paper. To Maria Schneider, Stephen Bent, Mayee Wong and Rodrigo Velazquez-Duran for technical assistance; the personnel of the Chamela Biological Station of the Instituto de Biología UNAM for logistic support during field work and; Heberto Ferreira and Alberto Valencia for their assistance in data processing. This project was supported by NASA NCC2-1051 and the Consejo Nacional de Ciencia y Tecnología, México CONACyT-G27674-N.

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