00387

UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

# POSGRADO EN CIENCIAS BIÓLOGICAS

Centro de Investigaciones en Ecosistemas

# ECOLOGÍA MICROBIANA Y DINÁMICA DEL C Y N EN SUELOS DE UN BOSQUE TROPICAL ESTACIONAL

# T E S I S

QUE PARA OBTENER EL GRADO ACADÉMICO DE

**DOCTORA EN CIENCIAS**

P R E S E N T A

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MÉXICO, D. F. NOVIEMBRE, 2005.

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Por medio de la presente me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 12 de septiembre del 2005, se acordó poner a su consideración el siguiente jurado para el examen de DOCTORA EN CIENCIAS de la alumna **NOGUEZ GÁLVEZ ANA MARÍA** con número de cuenta **76302291** y número de expediente **53772**, con la tesis titulada: **"Ecología microbiana y dinámica del C y N en suelos de un bosque tropical estacional"**, bajo la dirección del **Dr. Felipe Francisco García Oliva.**

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Dr. Juan Núñez Farfán
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**AGRADECIMIENTOS**

Agradezco al CONACyT por la beca otorgada para realizar mis estudios de doctorado. El trabajo de campo y laboratorio de esta tesis fueron financiados a través del Programa de Apoyo al Posgrado (PAEP, 201321), los Proyectos "CONACYT-G27674-N" y "NASA NCC2-1051".

Agradezco a los miembros de mi Comité Tutoral: Dr. Felipe García-Oliva, Dra. Irma Rosas Pérez y Dr. José Manuel Maass Moreno por su constante apoyo a lo largo del desarrollo de la tesis y sus múltiples consejos y enriquecedoras discusiones. Agradezco también a todos los miembros del jurado cuyos comentarios y sugerencias contribuyeron considerablemente para mejorar este trabajo.

La Dra. Valeria Souza y el Dr. Luis Eguiarte, aunque nunca formaron formalmente parte de mi Comité Tutoral, fueron parte esencial en el desarrollo de esta tesis, agradezco su apoyo en todo momento y el espacio que me otorgaron en su laboratorio en el cual realicé parte de los análisis moleculares. Los TRFLPs se realizaron gracias al apoyo del Dr. Larry Forney, jefe del Deparatamento de Ciencias Biológicas de la Universidad de Idaho, Moscu, ID, EUA.

Agradezco a Maribel Nava Mendoza su apoyo con los análisis químicos; a Heberto Ferreira su ayuda en el manejo de los datos; a Laura Espinosa, Ana Escalante y María Schneider por su ayuda con las técnicas moleculares; y a Salvador Araiza y Abel Verduzco por su apoyo en el trabajo de campo, y en general al personal de la Estación de Biología Chamela de la UNAM por su apoyo logístico durante el trabajo de campo.

Finalmente, quiero agradecer a mi familia: a Héctor por su apoyo incondicional y por ser un ejemplo a seguir en todo momento, a Ángela y Carolina por mantenerme divertida y ocupada y a mis padres por su constante ayuda.

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## RESUMEN

En esta tesis se analizaron: la dinámica del C y N en macro- y micro-agregados del suelo, el papel de la estructura de las comunidades microbianas en los procesos funcionales del suelo, en las dos fracciones de agregados, y la distribución espacial de las comunidades microbianas del mismo, a una escala local.

Los resultados indican que la dinámica del C y N en macro- y micro-agregados es diferente, encontrándose un tipo de C menos procesado y en menor concentración en los macro-agregados. Asimismo, el nitrógeno presenta una redistribución diferencial entre los dos tamaños de partículas, siendo mucho más estable en los macroagregados.

En relación a las comunidades microbianas, éstas son de naturaleza diferente en los dos tamaños de agregados, así como los procesos de transformación de nutrientes que realizan dentro de cada fracción. Los micro-agregados poseen una mayor proporción de organismos heterótrofos que estimulan la inmovilización del nitrógeno, protegiéndolo de su pérdida por lixiviación en la época más húmeda. En contraste, en los macro-agregados dominan las bacterias nitrificantes.

Con respecto a la distribución espacial encontramos que a una escala local los organismos estudiados presentan rangos de distribución reducidos con un alto porcentaje de "endémicos"; valores de diversidad ß entre 1-78 -1.9, los cuales son mayores a los que originalmente se asumían para estos organismos; una curva "especie-área"; en la que el número de "especies" se incrementa con el área muestreada y comunidades estructuradas localmente.

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**ABSTRACT**

In this work we conducted an investigation about C and N dynamics in soil macro- and micro-aggregates; the role of soil microbial community structure in these dynamics; and the spatial distribution of soil microbial communities at a local scale.

We found that soil C & N dynamics and transformation are different within soil macro- and micro-aggregates. Macro-aggregates posses lower concentrations of C than micro-aggregates and of less processed nature. There is also a differential N redistribution between the two soil fractions, being the N within macro-aggregates, more stable than in micro-aggregates.

Microbial communities present in the two soil fractions are of different nature and perform different nutrient transformation processes. Micro-aggregates present a greater proportion of heterotrophic organisms which estimulate N immobilization, avoiding its lost through lixiviation during the wettest period of the year. In contrast, within macro-aggregates nitrifying bacteria are dominant.

In the case of microbial communities, we found highly structured species assemblages, which showed high levels of beta diversity and a non-random nested pattern of diversity. We demonstrate a non-ubiquitous dispersal for soil prokaryotes, which suggest a complex biogeography similar to that found for terrestrial vertebrates.

# CAPÍTULO I

# INTRODUCCIÓN GENERAL

## Las Bacterias y Los Ecosistemas

Las bacterias juegan un papel relevante en la regulación de la circulación de nutrientes, el flujo de energía y la productividad primaria en los ecosistemas, tanto terrestres como acuáticos (Paul & Clark, 1989; Buckley & Schmidt, 2001; Hill *et al.*, 2000; Wardle et al. 2004). Debido a su diversidad metabólica estas pueden sobrevivir en prácticamente todos los ambientes existentes, incluyendo los extremos (Fulthorpe, et al. 1998; Ward *et al.* 1998; Nusslein & Tiedje 1999; Staley. & Gosink, 1999; Newman & Banfield 2002; Papke et al 2003). Asimismo, constituyen el grupo viviente con mayor diversidad tanto fisiológica como genética (Woese, 1987).

Actualmente existe la controversia sobre la existencia de patrones de distribución y diversidad en las comunidades bacterianas, similares a los que presentan los organismos macroscópicos (Finlay, 2002). Debido a su carácter unicelular, tamaño reducido y extraordinaria abundancia, ha persistido la idea de que las bacterias carecen de restricciones para su dispersión y por tanto de barreras geográficas o patrones de distribución, ya que se piensa, que por razones puramente estadísticas presentan altas tasas de migración y bajas tasas de especiación y extinción (Finley et al 1999; Finley 2002). Sin embargo, estas aseveraciones están basadas en la extrapolación de datos referentes únicamente a protozoarios ciliados.

La aparición de nuevas técnicas moleculares ha permitido empezar a explorar y tratar de encontrar respuestas sobre la historia evolutiva de los microorganismos, el ensamblaje de sus comunidades, su diversidad, actividad y abundancia (Hugenenholtz et al. 1998; Tiedje et al 1999; Ranjard et al. 2000; Curtis et al. 2002). Más aun las técnicas moleculares nos han dado las herramientas para empezar a discernir el papel de la diversidad de especies en los ecosistemas (Schimel & Gulledge 1998; Cavigelli & Robertson 2000, Prosser, 2002; Jessup et al. 2004).

Conocer la distribución espacial de las bacterias y los mecanismos que la regulan nos darán las herramientas necesarias para entender la redundancia funcional, las limitaciones en su dispersión y nos aportará datos potenciales

sobre el intercambio de rasgos ecológicamente relevantes. Como mencionan Curtis & Sloan (2004), si discutimos que la diversidad importa, entonces el estudio de los patrones de diversidad global arrojará información substancial a las investigaciones que buscan relacionar la función y la estructura en las comunidades.

## Los Suelos y el Bosque Tropical Estacional

La fuente principal de nutrientes disponibles en los suelos del bosque tropical estacional (BTE) se obtiene a partir de la mineralización de la materia orgánica (Singh et al. 1989; Raghubanshi et al. 1990; Srivastava 1992; Prasad et al. 1994; Campo et al. 1998; Giardina et al. 2000). La acumulación de formas disponibles de nutrientes en el suelo del BTE sucede durante el periodo de secas (Roy y Singh 1995; Singh y Singh 1991; Jaramillo y Sanford; 1995 Zarco Arista 2001) cuando la mayoría de los árboles carecen de hojas y la absorción de nutrientes por parte de las plantas, se encuentra abatida (Roy y Singh 1995; Campo et al 1998). Durante el periodo de lluvias se presenta un mayor movimiento de nutrientes entre los diferentes compartimentos del ecosistema, encontrándose en el suelo los valores más bajos de formas inorgánicas y microbianas (Zarco Arista 2001).

El contenido de agua en el suelo y su consecuente efecto sobre las poblaciones microbianas y procesos de mineralizacion, también se ve afectado por la variación en las características de las geoformas, definidas a través de su elevación, orientación, pendiente y material parental (Kachanoski 1988; Swanson et al. 1988; Raghubanshi, 1992; Silver et al. 1994; Tiessen et al. 1994). La variación en la disponibilidad de agua en el suelo en relación con los gradientes topográficos produce valores diferenciales en el almacén de nutrientes y la materia orgánica del suelo, especialmente en la dinámica del C, N y P (Silver et al. 1994; Burke et al. 1995; Morris & Boerner 1998).

La heterogeneidad topográfica a lo largo de la costa mexicana del Pacífico determina que en la región existan innumerables cuencas, en el caso particular de la estación de Biología Chamela, Jalisco, las cuencas hidrográficas

existentes se pueden caracterizar por su tipo, clase, rasgos y procesos de relieve (López-Blanco et al. 1999). Al interior de estas cuencas, existen laderas con diferentes características morfológicas: intervalos de pendiente, longitud, forma y orientación, que le dan a los suelos un patrón espacial diferencial en el contenido de nutrientes, contenido de humedad e incidencia de radiación solar (Martínez-Yrízar & Sarukhán 1990, 1993, Martínez-Yrizar et al. 1996; Galicia. et al. 1995; López Blanco et al. 1999; Zarco-Arista, 2001).

En el caso de los suelos del BTE de Chamela la cubierta vegetal y la dinámica de nutrientes en el ecosistema son esenciales para la conservación de la calidad del suelo. La proporción de macroagregados, en los suelos de este bosque, constituye aproximadamente el 80% de su masa total y juega un papel importante en el almacenamiento de carbono a corto y largo plazo (Garcia-Oliva et al., 1999; Garcia-Oliva & Tapia, 2001). Garcia-Oliva *et al.* (1999) observaron que después del primer ciclo del cultivo, siguiente a la transformación del bosque por el método de "roza-tumba-quema", se presentaba una drástica reducción en la proporción macro-agregados/micro-agregados, al igual que una disminución en los almacenes de C asociados con los macro-agregados. La información acerca del papel funcional de lo macro- y micro-agregados en este tipo de bosque es prácticamente inexistente al igual que la dinámica del C y N en ambos tamaños de partícula y los microorganismos asociados a éstas.

OBJETIVOS:

1. Establecer el papel funcional de macro- y micro-agregados del suelo en la dinámica del C y N en tres condiciones diferentes de humedad y contenido de materia orgánica.
2. Analizar el patrón de distribución de las comunidades bacterianas en las dos fracciones de agregados del suelo y en diferentes condiciones de humedad y relacionar dicho patrón con la dinámica del C y N.
3. Determinar el patrón de distribución espacial, a escala local, de las comunidades bacterianas del suelo a través del escalamiento de su

diversidad y el análisis de sus patrones biogeográficos en comparación con otros grupos.

## HIPÓTESIS GENERALES

1. La dinámica del C y N será diferente entre los dos tamaños de agregados y a su vez estará afectada por las condiciones edáficas en cada una de las posiciones del relieve.
2. La riqueza y estructura de las comunidades bacterianas del suelo serán diferente entre los tamaños de agregados, independientemente de la posición del suelo en el relieve.
3. Se espera que la distribución espacial de las comunidades bacterianas tenga una estructura no aleatoria y que los valores de diversidad ß sean altos.

## ZONA DE ESTUDIO

El trabajo se realizó en la Estación de Biología Chamela, dentro del marco del proyecto a largo plazo "Estructura y funcionamiento de un ecosistema tropical estacional en Chamela, Jalisco" México, localizada entre los 19°29' – 19°34' latitud norte y los 104°58' -105°04' longitud oeste. El clima es $Aw_{0i}$, cálido sub-húmedo, con lluvias en verano (García-Oliva et al. 2003). Presenta un régimen isotermal, con una temperatura media anual de 24°C. El 80% de la precipitación se concentra en los meses de julio a octubre (Bullock 1986) con una precipitación media anual acumulada de 788mm (promedio de 1977 a 2000; García-Oliva et al. 2003). La vegetación dominante es la Selva Baja Caducifolia (Rzedowski 1978) o bosque tropical estacional (BTE), el cual se caracteriza por un patrón estacional de lluvias, en donde la disponibilidad de agua es el factor determinante de la estructura vegetal y del flujo de nutrientes en el ecosistema (Lugo y Murphy 1986; Mooney *et al.* 1995; Holbrook *et al.* 1995). La vegetación responde a los cambios en la disponibilidad de recursos en forma estructural, fisiológica y fenológica, siendo tal vez esta última la más evidente de todas (Holbrook et al., 1995).

Generalmente, estos bosques presentan una composición más simple y una estructura menor que los bosques más húmedos que se encuentran en la región tropical, siendo sin embargo muy diversos (Murphy y Lugo, 1995).

- Problemas de deforestación

El sistema agrícola tradicional de roza-tumba-quema, el desarrollo de agricultura mecanizada y el establecimiento de praderas para agostadero, se encuentran entre las principales causas de desaparición del BTE, que hasta hace unos años ocupaba más de la mitad de los trópicos del mundo (Challenger 1998; Janzen, 1988). En México el BTE constituía el 60% de la vegetación tropical del país, porcentaje que se ha ido reduciendo drásticamente. Challenger en 1998 reportaba que de la cobertura original del BTE en México únicamente se mantenía entre el 31% y el 45%. Sin embargo, un estudio más reciente y detallado elaborado por Dirzo y Trejo (2000) sugiere que ya para el inicio de la década de los noventa únicamente se conservaba el 27% del BTE, principalmente en manchones aislados en la partes bajas de la Sierra Madre Occidental, en Sonora y Sinaloa, Jalisco, Tehuacán-Cuicatlán, sur de Tamaulipas y Querétaro.

Las tasas de deforestación, 2.2 % año$^{-1}$ (Masera et al. 1997), y la constante presión económica y social del país amenazan continuamente la integridad de este bosque (Challenger 1998). La desaparición de la cubierta vegetal genera entre otros problemas: la pérdida de la biodiversidad y del pool genético, la perturbación del régimen hidrológico, la pérdida de los mecanismos encargados de la conservación de nutrientes en el ecosistema, y la pérdida de la calidad del suelo (Soulé & Kohm 1989; Wilson 1988; Maass 1995).

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# CAPÍTULO II

# SOIL C AND N DYNAMICS WITHIN TWO SOIL AGGREGATES SIZE-FRACTIONS IN A TROPICAL DECIDUOUS FOREST

Cover Page

Type of contribution

Date of preparation, number of text pages, number of tables, figures, etc.

Title: Soil C and N dynamics within two soil aggregates size-fractions in a tropical deciduous forest

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**Abstract**

Soil aggregates play and important role in soil structure dynamics and C sequestration in ecosystems like the tropical dry forest (TDF) in the western coast of Mexico, this function is particularly important because the high proportion that aggregates represent of the total soil mass (80%). After forest transformation, to pasture or agriculture, all the mechanisms sustaining aggregate formation and maintenance are destroyed. The extent at which aggregates of different sizes contribute to the preservation of soil structure and the biogeochemical dynamics of C and N is a question that has not been answered for the tropical soils. Our purpose was to assess the functional role of micro-aggregates and macro-aggregates in C and N dynamics in soils with three different conditions of humidity and soil organic matter in a TDF in the state of Jalisco, Mexico. Micro-aggregates had significantly higher nutrient concentrations than macro-aggregates, and the ratio at which C was processed, was faster in micro-aggregates than in macro-aggregates.

**Key words**: C mineralization, macro-aggregates, micro-aggregates, microbial activity, Mexico, nitrification.

## 1. Introduction

Soil organic matter (SOM) distribution and turnover rates are highly related to soil aggregate dynamics (Elliot 1986; Six et al. 2004). In turn, the role that soil aggregates play in soil structure stability, soil organic carbon (SOC) protection, nutrient availability, and microbial activity is critical for soil integrity maintenance (Beare et al. 1994b; Six et al. 2000, 2002a; Denef et al. 2001a; García-Oliva et al. 2004). The sequential formation of primary particles into micro-aggregates (<250 μm) that in turn form macro-aggregates (>250 μm) involves organic binding agents of different nature and stability (Tisdall and Oades 1982). Macro-aggregates are bound by temporary, as well as transient agents, while micro-aggregates by persistent elements (Tisdall and Oades 1982, Oades 1993). Macro-aggregate formation starts with the entangling of fine particles via fine roots and hyphae, coupled with the action of cementing agents formed as by-products of the fresh organic matter decomposition and by exudates from the same roots and hyphae (Ashman et al. 2003; Garcia-Oliva & Tapia 2001; Six et al. 2002b, 2004). The intra particulate organic matter (iPOM) held within the macro-aggregates is decomposed into finer POM which is then combined with metals, giving origin to micro-aggregates within the macro-aggregates (Jastrow 1996; Six et al. 1998, 2002b). Therefore, micro-aggregates are protected within the stable macro-aggregates (Elliott 1986; Beare et al. 1994a; Gupta and Germida 1988) and when the latter break the former are released along with a liberation of labile SOM, enhancing microbial activity, which in turn depletes SOM storage altering the structural stability of the soil (Elliott et al. 1986).

Although aggregate formation and disruption is a natural process, it is well documented that upon forest conversion, for cultivation or pasture, the aggregate cycle is interrupted followed by a rapid lost of soil structure and fertility (García-Oliva et al. 1999; Spaccini et al. 2001). Kushwaha et al. (2001) found, for a tropical dryland agro-ecosystem, that in soils transformed by traditional tillage cultivation, the labile and the inter- micro-aggregate organic matter are lost, reducing the long term fertility of the soil. In contrast, when

these soils used for cultivation with moderate or zero tillage, an important increment in aggregate stability and C and N concentrations were observed for the macro- and micro-aggregates fractions of the soil.

In the tropical deciduous forest (TDF) of the western coast of Mexico the proportion of macro-aggregates found in the forest soils constitutes about 80% of the total soil mass playing an important role in the short and longtime C storage (Garcia-Oliva et al., 1999; Garcia-Oliva & Tapia, 2001). A significant cutback in the soil C pools associated with macro-aggregates, as well as a drastic reduction in macro-/micro-aggregates ratio occur in this forest, following its transformation by the traditional "slash and burn" practice (Garcia-Oliva et al., 1999). Considering the importance as a C storage pool and the rate of conversion of this forest (Houghton et al. 1991; Trejo and Dirzo 2000), the main purpose of our work was to assess the functional role of macro-aggregates and micro-aggregates in the C and N dynamics in soils within three different landscapes positions (top-hill; south-facing mid-slope and; north-facing mid-slope), which present different conditions of soil humidity, soil organic matter contents and productivity; and during three different periods during the year (dry season, onset of the rainy season, and rainy season).

## 2. Materials and Methods

### *2.1 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 (Sarukhán & Maass 1990; Maass et al. 2002). Predominant landscape forms are low hills with steep slopes (< 20°). Rhyolites from the Tertiary period are the main soil parent material and kaolinite is the dominant clay mineral (Campo et al. 2001). Soils are sandy clay loams, poorly developed with a pH of 6.9 (García-Oliva & Maass 1998), and according to the USDA system they are Typic Ustorthents (Cottler et al. 2002).

Soil organic carbon (SOC) and soil organic nitrogen (SON) mean concentrations are 32 mg C $g^{-1}$ and 2.2 mg N $g^{-1}$, respectively (García-Oliva *et al.* 2003). Around 30% of soil organic matter (SOM) is concentrated in the 0-5 cm depth (García-Oliva & Maass 1998) and about 76% of the fine root productivity in the forest soils occurs in the first 5 cm (Castellanos *et al.* 2001). The mean annual *temperature is 24.6 °C and the mean annual precipitation is (García-Oliva et al.* 2002). Approximately 50 % of the annual precipitation is 788 mm (Garcia-Oliva et al. 2003), usually fetched in by less than six highly erosive tropical rainstorms (García-Oliva *et al.* 1995). Rains last generally from June to October, being September the wettest month (García-Oliva *et al.* 2002). The dominant vegetation is tropical deciduous forest (TDF), where most of the species are leafless seven months during the year (Bullock and Solís–Magallanes 1990). Total net primary productivity averages 12 Mg dry matter $ha^{-1}$ $yr^{-1}$ (Martínez-Yrízar *et al.* 1996).

*2.2 Field sampling*

We used a nested designed with three factors: three landscape positions (south-facing mid-slopes, north-facing mid-slopes and top-hills); two soil aggregate size fractions (micro-aggregates < 250 μm and macro-aggregates > 250 μm) and; three sampling dates: dry season (DS; May), onset of rainy season (ORS; June) and rainy season (RS; September) (Figure 1). This design was used in order to be able 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 have different solar radiation indexes (SRI; north aspect: 3651 MJ $m^{-2}$ $yr^{-1}$; south aspect 4475 MJ $m^{-2}$ $yr^{-1}$; top-hill: 4273 MJ $m^{-2}$ $yr^{-1}$; Galicia et al. 1999) and different soil nutrient and water contents (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). In each plot and in each sampling date composite samples, of 15 undisturbed topsoil (0-5 cm), were randomly taken. These samples were stored in black plastic bags and kept in refrigeration at 4° C, until their use in the laboratory. Sampling was conducted in the year 2002 at three different dates during the year: the dry season (DS), the onset of the rainy season (ORS) and, the rainy season (RS).

*2.3 Soil analyses*

Prior to the biogeochemical analyses, soil samples were dry-sieved, in order to avoid labile carbon and inorganic nitrogen lost; dry separated in two aggregate size fractions, micro-aggregates (< 250 μm) and macro-aggregates (> 250 μm), small pebbles and sand material were excluded manually. Before the total nutrient forms analyses, samples were ground with a 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 Indutrial System 1977) and colorimetric readings were done using an auto-analyzer (Bran+Luebbe 3 Auto Analyzer, Germany).

In field-moist samples, microbial C (Cm) and N (Nm) were determined 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 the 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 then 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, inorganic N, $NH_4^+$ and $NO_3^-$, was extracted from 5 g soil sub-samples by shaking for 30 minutes 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 diameter) with a 0.17 mm mesh at the bottom. Samples were incubated in 1 L jars for 15 days at 25 °C and 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.

*2.5 Statistical analyses.*

Biogeochemical parameters. The effect of the three factors considered (position, aggregate size and time) on nutrient concentration was analyzed by a repeated-measures MANOVA, in those cases where the results were expressed as rates or ratios we performed logarithmic transformations. When the esphericity test was significant ($p<0.05$) we used the Greenhouse-Geisser analysis in order to adjust the F value. We had a nested design, where time was nested in aggregate size which in turn was nested landscape position. 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 time factor (DS, ORS and, RS). When the results obtained were significantly different we used

orthogonal contrasts to find out which factors were different, these tests were performed using JMP program (SAS Institute, Inc. 1995). We also performed Pearson correlation analyses, separately for each aggregate size, in order to find any linear relationship among the variables measured for each soil fraction (n=21). We ran stepwise regressions, for each soil fraction, to find any possible relationship between the potential C mineralization rate ($C\text{-}CO_2$) with the following variables: labile C, microbial C, microbial N, $NH_4^+$, $NO_3^-$ and total C/N ratio contents. These analyses were done by using Statistix 7 package (Analytical Software, 2000).

## 3. Results

### *3.1 Total forms*

Total Carbon. None of the interactions were significant ($P>0.05$); however, C values were significantly different ($P<0.05$) for the three factors considered (Table 1). North-facing mid-slopes presented the highest total C contents, independently of the date and the aggregate size. In general, micro-aggregates presented higher C concentrations than macro-aggregates within the three sampling dates and the three positions considered (Fig. 2a). With the exception of macro-aggregates in the North-slope, the dry season showed the highest concentration of total C.

Total Nitrogen. Total N concentrations were significantly higher in the north-facing mid-slope than in the other two positions ($P<0.05$; Table 1).The interaction time*size was significant ($P<0.05$; Table 1). N values were higher within micro aggregates than within macro-aggregates; however, N tendencies within the three dates considered were different between the two size fractions, N dynamic within micro-aggregates was affected by seasonality (cumulative rainfall), within micro-aggregates N decreased with time, whereas within macro-aggregates concentrations showed no changes (Fig. 2b).

Carbon/Nitrogen. There was no position effect on the C/N ratio ($P>0.05$; *Table 1*).The interaction time*size was significant ($P<0.05$; *Table 1*). There was a significant difference in C/N ratio with time ($P<0.05$), values tend to decrease

slightly in macro-aggregates and to increase in micro-aggregates, in the DS and at the ORS macro-aggregates presented higher values than micro-aggregates (Fig. 2c).

*3.2 Available and Microbial Forms*

Labile Carbon ($C_{labile}$). There was not a significant effect of position in $C_{labile}$ ($P > 0.05$; Table 1), but there was a significant interaction between time*size ($P<0.05$; Table 1), $C_{labile}$ concentrations were higher in the DS and at the ORS for both size fractions; however, in micro-aggregates there was a reduction trend in $C_{labile}$ concentration with time; whereas in macro-aggregates had similar concentrations in the DS and at the ORS (Fig. 3a).

Ammonium ($NH_4^+$). Differences in $NH_4^+$ concentration due to position were not significant ($P > 0.05$; Table 1). There was a significant interaction between time*size ($P<0.05$). There was a tendency, in $NH_4^+$ concentration, to increase with time in all but micro-aggregates in the north position (Fig. 3b). There was also a higher $NH_4^+$ concentration in micro-aggregates than in macro-aggregates.

Nitrates ($NO_3^-$). There was no effect of position in $NO_3^-$ concentration ($P > 0.05$: Table 1). There was a significant interaction time*position ($P<0.05$) and also time*size ($P<0.05$; Table 1). During the DS and the ORS $NO_3^-$ concentrations were very similar; however, there was an important increment in $NO_3^-$ concentration with time, for the three positions and both sizes of aggregates (Fig. 3c). In the RS $NO_3^-$ increased more than three times the concentration of the previous sampling dates, and in the case of the north-slope the increments were higher. Micro-aggregates in the RS had higher $NO_3^-$ concentration than macro-aggregates.

Microbial Carbon. There was a significant effect of position ($P<0.05$; Table 1), microbial C concentrations, in the south-facing mid-slope, were lower than for the other two positions, with exception of micro-aggregates in May (Fig. 4a). The interaction time*size was significant ($P<0.05$; Table 1), samples from the

DS presented the highest values, in both size fractions, but concentration within micro-aggregates was higher than in macro-aggregates (Fig. 4a).

Microbial Nitrogen. Although there was no significant difference among the three positions (*P>0.05*; Table 1), the interaction time*position was significant (*P=0.05*; Table 1). Microbial N concentrations were higher for the DS than for the ORS and RS, in which concentrations were very similar for both fraction sizes; however, concentrations for micro-aggregates were higher than in macro-aggregates, especially in the ORS where the concentration was almost four times higher than in macro-aggregates (Fig. 4b).

Microbial C:N ratio. There was not significant effect of any of the three factors considered (time, position and aggregate size; *P>0.05*; Table 1).

*3.5 Potential Mineralization*

$CO_2$-C. There was no position effect on the $CO_2$-C evolved during the incubations (*P>0.*05: Table 1). However, the interaction time*size was significant (*P<0.05*). Soils from the DS had a greater $CO_2$-C production, for both size particles, this $CO_2$-C production decreased drastically in the samples from the rainy months (June and September; Fig. 5). In the case of micro-aggregates $CO_2$-C production was higher than in macro-aggregates for the ORS and the RS in the three positions considered, and also in the DS in the north-slope (Fig. 5).

*3.6 Pearson correlation & Stepwise regression*

Pearson correlation. This analysis showed, for macro-aggregates, positive and significant relationships (*P< 0.*005) for $C_{labile}$ and $C_{microbial}$, $C_{total}$, $N_{microbial}$, $N_{total}$; $C_{microbial}$ and $C_{total}$, $N_{microbial}$; $C_{total}$ and $NH4^+$, $N_{microbial}$, $N_{total}$; $NH4^+$ and $NO_3^-$; $NO_3^-$ and $N_{total}$ (Tabla 2). There were also negative significant relations (*P< 0.*005) for $C_{labile}$ and $NO_3^-$; $C_{microbial}$ and $NH4^+$; and also for $C_t/N_t$ and $NO_3^-$ and $N_{total}$ (Table 2).

In the case of micro-aggregates the positive and significant relationships ($P<$ 0.005) were among $C_{labile}$ and $C_{microbial}$, $C_{total}$, $N_{microbial}$, $N_{total}$; $C_{microbial}$ and $C_{total}$, $N_{microbial}$; $N_{total}$; $C_t/N_t$ and $C_{total}$, $NO_3^-$; $C_{total}$ and $N_{total}$; $N_{microbial}$ and $N_{total}$ (Tabla 2). Stepwise regression. We found the $CO_2$-C production, in the case of macro-aggregates, was related positively with $C_{microbial}$ and negatively with the $NO_3^-$, $NH_4^+$ and $C_{labile}$ found in the samples previous to the incubation **[$CO_2C = 53.96 - 0.0888\ C_{labile} + 0.44\ C_{microbial} - 1.59\ NH_4^+ - 0.384\ NO_3^-$; $r^2 = 0.77$; $P < 0.001$)**. In the case of micro-aggregates $CO_2$-C production was related positively with $C_{microbial}$ and negatively with $NO_3^-$ **($CO_2C = 59.1 + 0.019\ C_{microbial} - 044\ NO_3^-$; $r^2 = 0.53$; $P < 0.001$)**.

## 4. Discussion

Our results indicate that the influence of the position within the landscape is only reflected in the concentration of the total forms of C and N. This can be the expression of the differential net primary productivity found at the three locations (Martinez-Yrízar & Sarukhán 1990, 1993, Martínez-Yrizar et al. 1996), which in turn responds to the soil humidity gradient produced by the differential solar radiation received in these areas (Galicia et al. 1999). North-slope sites had higher above and below-ground productivity and lower water demand than the other two landscape positions (Martínez-Yrizar et al. 1996; Galicia et al. 1999). However, the differences between the two soil aggregates size-fractions were not affected by landscape position, therefore, the nutrient dynamics within aggregates size fractions are independent of landscape position.

We found, in general, significantly higher nutrient concentrations in micro-aggregates than in macro-aggregates, as observed in other studies where dry aggregate separation was employed (Ashman et al., 2002; Beare et al. 1994a). Micro-aggregates presented more labile and microbial C per unit of total C than macro-aggregates. There was also a differential labile C lost rate, being greater in micro-aggregates than in macro-aggregates. The reduction in total C with time was the result of the reduction in both labile C and microbial

C, which explain more than 60% of the reduction of total C. At the onset of the rainy season some of the labile C is lost by lixiviation and its transformation within micro-aggregates occurs very rapidly (Campo et al. 1998; García-Oliva et al. 2003), even though the microbial biomass content is very similar at this stage for both aggregate size fractions, and although labile C concentration is much greater in micro-aggregates than in macro-aggregates. This indicate that the organic matter present in macro-aggregates was less processed than that found in micro-aggregates; giving as a result on the one hand a much faster consuming rate in micro-aggregates than in macro-aggregates, and on the other a lower C and N long term storage capacity in micro-aggregates.

The total C/total N ratio indicates that C dynamic was different and more stable in macro-aggregates than in micro-aggregates, independently from the position studied. It is likely that the energy sources in micro-aggregates are constituted of microbial and soluble compounds, perhaps exo-polysaccharides, which are more easily degradable (Ashman et al. 2003). These results are consistent with the model proposed by several authors in which they state that the decomposition characteristics of the organic matter associated with macro- and micro-aggregates are different; the organic matter associated with macro-aggregates is less processed than that associated with micro-aggregates (Elliott 1986; Gupta and Germida 1988; Beare et al. 1994b; García-Oliva et al. 2003). In addition, the labile C present in macro-aggregates is not completely available because it is located within the micro-aggregates that integrate the macro-aggregates (Oades 1984; Elliott and Coleman 1988; Oades and Waters 1991). Therefore, the biogeochemical processes within each aggregate size-fraction are carried out at different rates. Our results suggest that micro-aggregates have higher rates than in macro-aggregates.

Microbial carbon reduction with time, from the dry to the rainy season, was also the result of the reduction in labile carbon, the main energy source of the heterotrophic microorganisms, as it is shown by the positive and significant correlation between microbial and labile carbon. This positive correlation also indicates that during the dry season there was enough energy for

microorganisms to carry out C mineralization processes. However, at the onset of the rainy season (June) micro-aggregates metabolize more rapidly the available C; at this time, redistribution and transformation of nitrogen started, being stronger during the rainy season.

Once more, nitrogen redistribution and lost between the two sizes of aggregates were very different; while the total nitrogen contents within macro-aggregates was very stable with time, the lost within micro-aggregates was very fast, and by the rainy season the amount of total N in this fraction was almost half that amount found during the dry season. With the reduction of the labile C sources, energy was obtained throughout the transformation of ammonium, dominating the nitrification and denitrification processes (Vitousek et al. 1982; Hart et al. 1994). Nitrification was dominant over ammonification in the rainy season; nitrate concentration was at least three times higher than that of $NH^{+}_{4}$. Similarly, a previous work performed in the same forest indicates that $N_2O$ fluxes were higher in the rainy season than in the dry season (García-Méndez et al. 1991).

Aggregate formation and disruption along a year cycle in this forest soils is very dynamic (Garcia-Oliva & Tapia, 2001), but it is maintained by the organic matter coming from the standing and surface litter, the soil biota and their exhudates production, and the proportion of soil particles (Garcia-Oliva et al. 2003). It is important to take into account the differential C & N transformation between macro- and micro-aggregates in this forest soils because the change in the proportion of these soil size fractions that occur after this forest transformation, could eventually lead to the reduction in C sequestration and the mechanisms that are strongly linked with the carbon pool protection (García-Oliva et al. 1999; Garcia-Oliva et al. 2003; Garcia-Oliva et al. 2004; Six et al. 1998; Six et al. 2000). Forest transformation leads not just to soil degradation, but to the drastic lost in the macro-aggregate size fraction and all the mechanisms that enable its formation. Micro-aggregates within macro-aggregates maintain the carbon renovation and storage, while

micro-aggregates outside macro-aggregates tend to enhance carbon lost and subsequently soil degradation.

Acknowledgments

The authors thank Salvador Araiza, Carlos Anaya and Manuel Montaño for their assistance during the field work; Leopoldo Valiente for his comments on the statistical analyses; Heberto Ferreira for his assistance in processing data; and the personnel of the Chamela Biological Station, *UNAM*, for providing us with the logistic support during the field work. This project was financed by *CONACyT*, México (Grant No. G27674-N).

Table 1. Probabilities and F values resulting of the repeated measures MANOVA.

| | Position | | Size(Position) | | Time | | Time*Position | | Time*Size(Position) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| C-total | 8.37 | 0.00* | 14.30 | 0.00* | 4.57 | 0.01* | 1.96 | 0.12 | 0.95 | 0.46 |
| N-total | 8.83 | 0.00* | 44.04 | 0.00* | 36.35 | 0.00* | 0.78 | 0.53 | 15.25 | 0.00* |
| Ct/Nt | 0.23 | 0.74 | 5.17 | 0.00* | 5.88 | 0.00* | 0.57 | 0.67 | 7.02 | 0.00* |
| C-labile | 2.19 | 0.12 | 10.69 | 0.00* | 78.05 | 0.00* | 1.58 | 0.19 | 8.11 | 0.00* |
| C-mic. | 5.74 | 0.00* | 19.37 | 0.00* | 414.03 | 0.00* | 4.47 | 0.01* | 4.34 | 0.00* |
| N-mic. | 1.29 | 0.28 | 13.09 | 0.00* | 85.59 | 0.00* | 3.02 | 0.05 | 10.32 | 0.00* |
| C/N (microbial) | 2.13 | 0.13 | 0.69 | 0.56 | 1.08 | 0.34 | 0.39 | 0.80 | 0.54 | 0.77 |
| $NO_3^-$ | 1.17 | 0.32 | 0.70 | 0.55 | 18.44 | 0.00* | 6.06 | 0.00* | 3.93 | 0.00* |
| $NH_4^+$ | 1.16 | 0.32 | 1.30 | 0.28 | 30.91 | 0.00* | 0.762 | 0.51 | 7.53 | 0.00* |
| $CO_2$-C | 5.74 | 0.00* | 19.37 | 0.00* | 333.24 | 0.00* | 2.23 | 0.07 | 6.91 | 0.00* |

Tabla 2. Pearson correlations among the different parameters measured, for macro-aggregates and micro-aggregates, separately.

Macro-aggregates (n= 21)

| | $C_{labile}$ | $C_{mic.}$ | $C_{total}$ | $C_t/N_t$ | $NH_4$ | $N_{mic.}$ | $NO_3$ |
|---|---|---|---|---|---|---|---|
| $C_{mic.}$ | 0.3401 | | | | | | |
| P | 0.0079* | | | | | | |
| $C_{total}$ | 0.3794 | 0.3211 | | | | | |
| P | 0.0028* | 0.0124* | | | | | |
| $C_t/N_t$ | 0.0397 | 0.0681 | 0.5323 | | | | |
| P | 0.7630 | 0.6051 | 0.0000* | | | | |
| $NH_4$ | -0.2521 | -0.2590 | -0.1066 | -0.1347 | | | |
| P | 0.0520 | 0.0457* | 0.4175 | 0.3047 | | | |
| $N_{mic}$ | 0.3558 | 0.8892 | 0.2875 | 0.0323 | -0.1144 | | |
| P | 0.0053* | 0.0000* | 0.0259* | 0.8064 | 0.3840 | | |
| $NO_3$ | -0.4477 | -0.2407 | 0.0875 | -0.2726 | 0.2552 | -0.1519 | |
| P | 0.0003* | 0.0639 | 0.5060 | 0.0351* | 0.0491* | 0.2466 | |
| $N_{total}$ | 0.3394 | 0.2376 | 0.6257 | -0.3030 | -0.0282 | 0.2347 | 0.4108 |
| P | 0.0080* | 0.0676 | 0.0000* | 0.0186* | 0.8306 | 0.0711 | 0.0011* |

Micro-aggregates (n=21)

| | $C_{labile}$ | $C_{mic.}$ | $C_t/N_t$ | $C_{total}$ | $NH_4$ | $N_{mic.}$ | $NO_3$ |
|---|---|---|---|---|---|---|---|
| $C_{mic.}$ | 0.5750 | | | | | | |
| P | 0.0000* | | | | | | |
| $C_t/N_t$ | -0.4997 | -0.3489 | | | | | |
| P | 0.0000* | 0.0059* | | | | | |
| $C_{total}$ | 0.3478 | 0.3730 | 3048 | | | | |
| P | 0.0060* | 0.0031* | 0.0169* | | | | |
| $NH_4$ | -0.1807 | -0.1294 | 0.1157 | -0.1062 | | | |
| P | 0.1634 | 0.3202 | 0.3744 | 0.4153 | | | |
| $N_{mic}$ | 0.4732 | 0.8972 | -0.3428 | 0.1664 | -0.1377 | | |
| P | 0.0001* | 0.0000* | 0.0068* | 0.2001 | 0.2901 | | |
| $NO_3$ | -0.6992 | -0.2816 | 0.5777 | 0.0260 | 0.1972 | -0.2486 | |
| P | 0.0000* | 0.0279* | 0.0000* | 0.8426 | 0.1276 | 0.0533 | |
| $N_{total}$ | 0.7159 | 0.7184 | -0.6657 | 0.3968 | -0.1272 | 0.5586 | -0.5228 |
| P | 0.0000* | 0.0000* | 0.0000* | 0.0015* | 0.3286 | 0.0000* | 0.0000* |

Figure 1. Precipitation and soil moisture from June to December 2002; in the figure we can observe the changes in soil moisture in relation to the amount of precipitation.

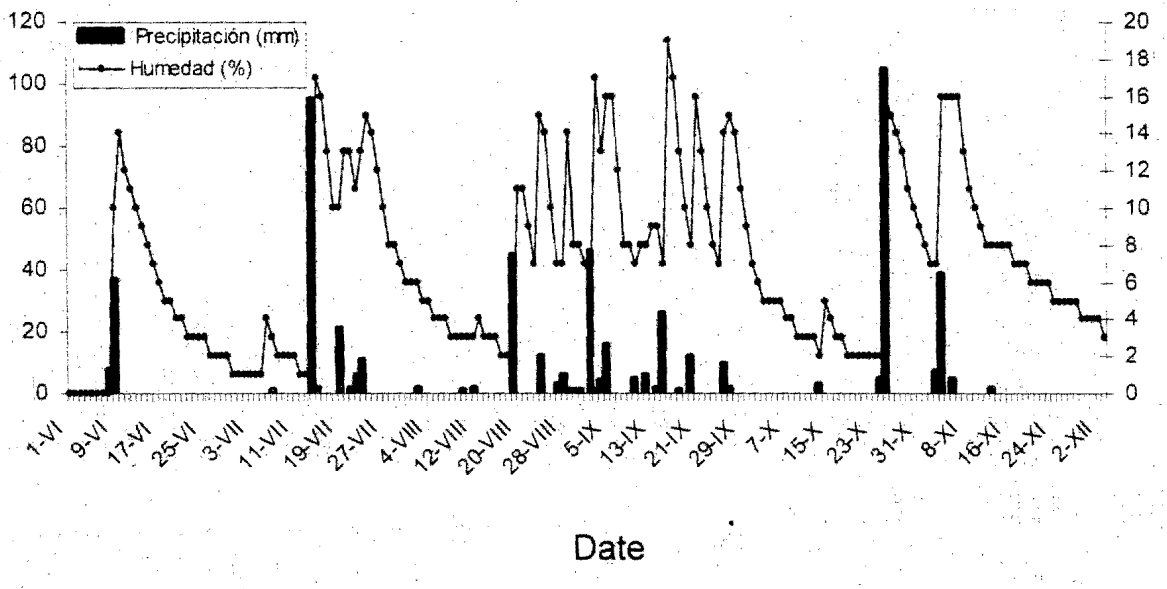


Figure 2. Mean values of (a) total carbon; (b) total nitrogen and (c) total C/N ratio concentrations in soil macro-aggregates (Ma) and micro-aggregates (Mi), at three different landscape positions, and in three different periods of the year (dry season-DS; onset of the rainy season-ORS ; and rainy season-RS)

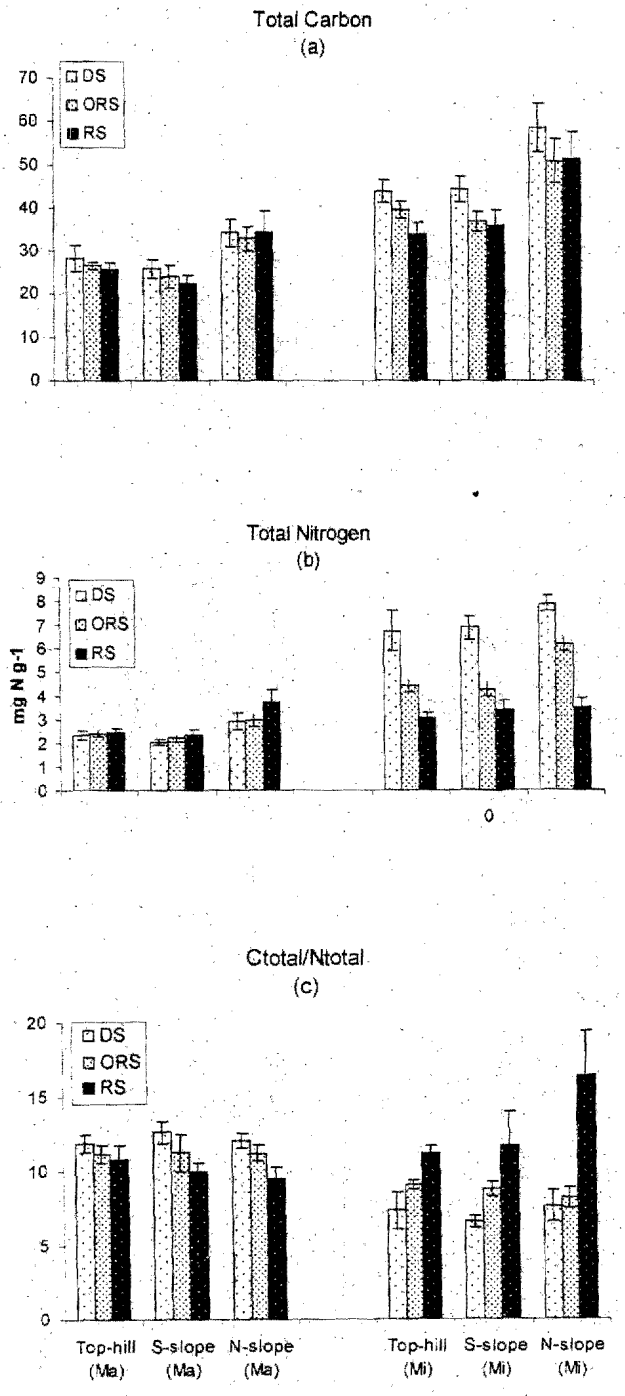


Figure 3. Mean values of (a) labile C; (b) $NH_4$; and (c) $NO_3$ concentrations in soil macro-aggregates (Ma) and micro-aggregates (Mi), at three different landscape positions, and in three different periods of the year (dry season-DS; onset of the rainy season-ORS; and rainy season-RS)

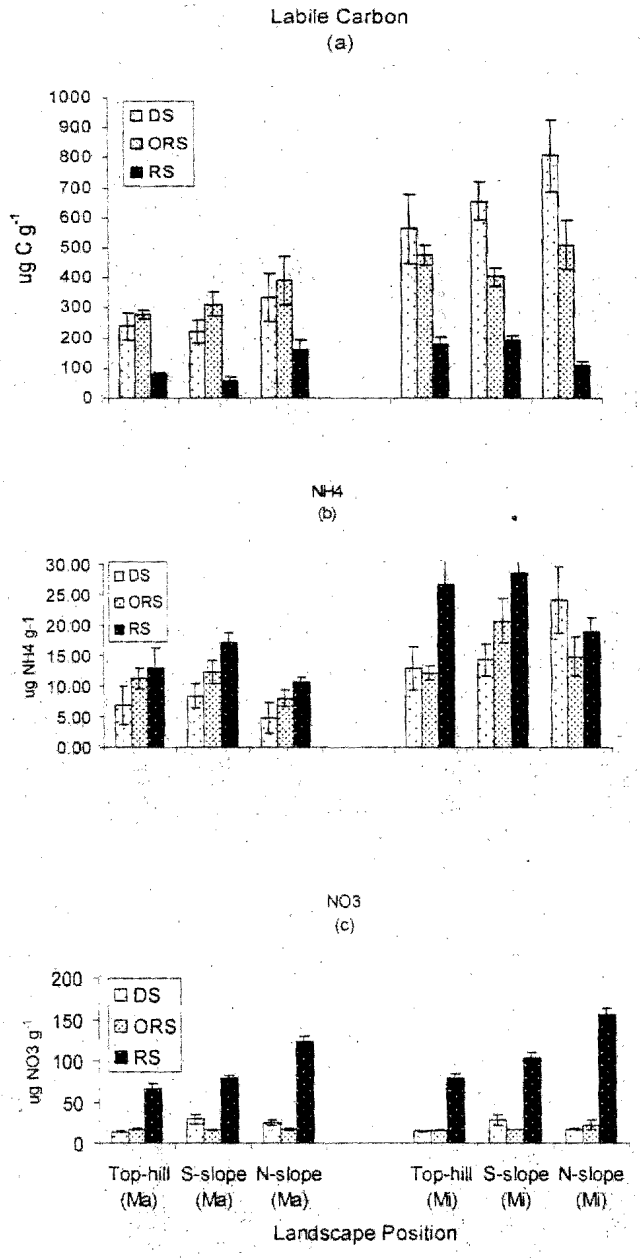


Figure 4. Mean values of (a) microbial C; (b) microbial N; and (c) microbial C/N ratio in soil macro-aggregates (**Ma**) and micro-aggregates (**Mi**), at three different landscape positions, and in three different periods of the year (dry season-**DS**; onset of the rainy season-**ORS**; and rainy season-**RS**)

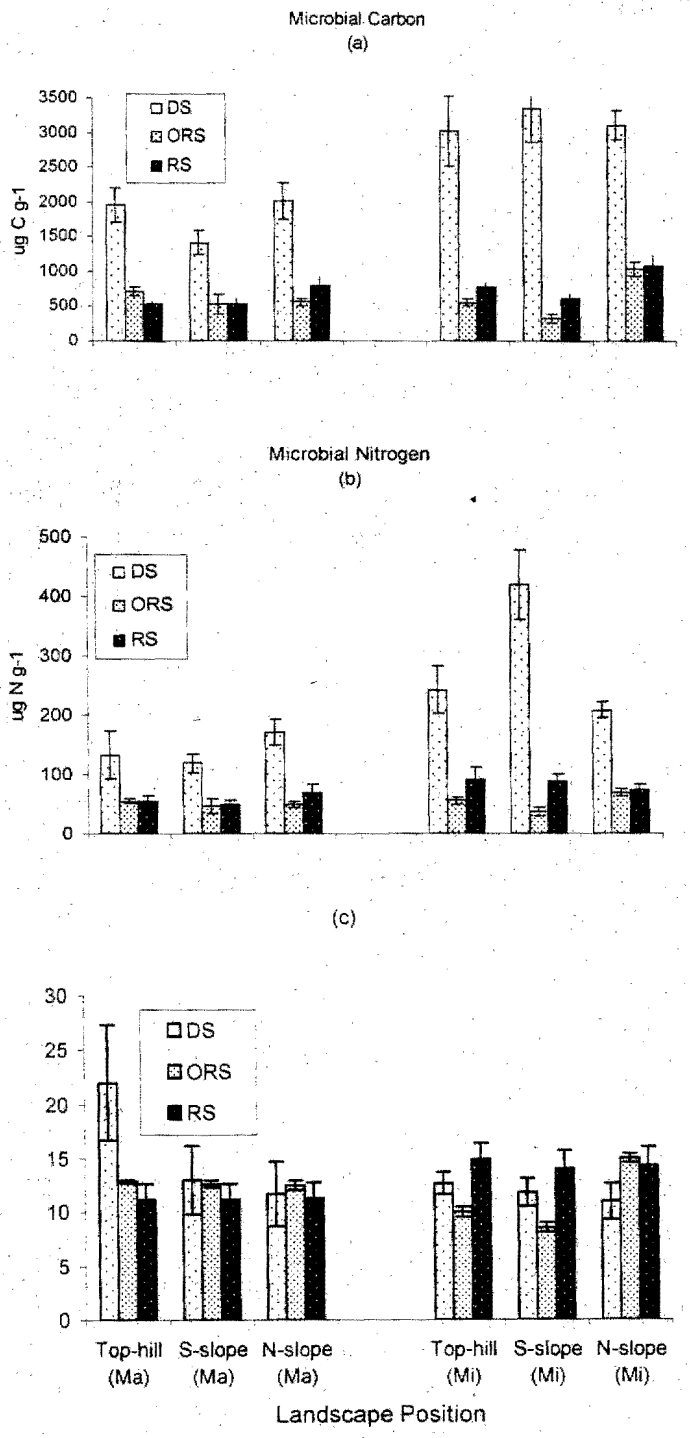
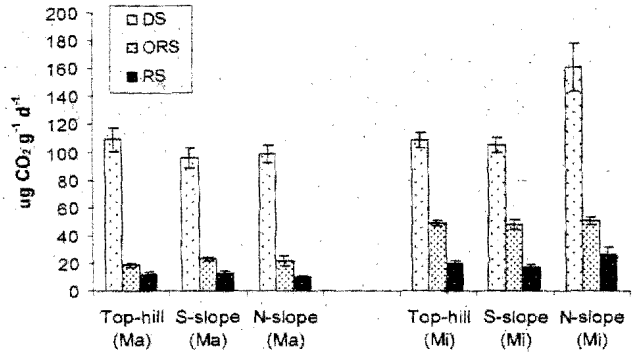


Figure 5. Mean values of $CO_2$-C production in soil macro-aggregates (**Ma**) and micro-aggregates (**Mi**), at three different landscape positions, and in three different periods of the year (dry season-**DS**; onset of the rainy season-**ORS**; and rainy season-**RS**)



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# CAPÍTULO III

# SOIL AGGREGATES AND MICROBIAL COMMUNITIES IN A TROPICAL DECIDUOUS FOREST IN WESTERN MEXICO

Soil aggregates and microbial communities in a tropical deciduous forest in western Mexico

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**Abstract**

We measured microbial community structure and C and N dynamics within soil macro- and micro-aggregates, in a tropical deciduous forest of the western coast of Mexico. We found that the biogeochemical processes that take place within the two soil aggregate sizes are of different nature and are also carried out by different microbial communities. Moreover, the less abundant organisms are those that perform the different processes within each soil fraction.

**Key words**: macro-aggregates, micro-aggregates, TRFLPs, soil microbial communities, carbon, nitrogen.

**Running title**: Soil microbial communities

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## 1. Introduction

The role played by microbial communities is crucial in the regulation of nutrient cycling, energy flow and primary productivity in terrestrial ecosystems (Paul and Clark 1989; Buckley and Schmidt 2001). Evidence has been found recently about the non-random distribution of bacteria in soils that affect the different processes they carry out (Norris et al, 2002; Nunan et al. 2003; Grundmand, 2004). The different aggregate fractions found in the soil environment set an intrinsic heterogeneity that affects microorganisms distribution (Mendes and Bottomley 1998, Mendes et al. 1999; Ettema and Wardle 2002). Grundman and Dubouzie (2000) suggest that aggregates and fine roots influence *Nitrobacter* aggregation and distribution in soils. There is also evidence that microbial biomass varies among different sizes of aggregates because of their differential physical and chemical characteristics, which favours the differential colonisation of microorganisms in soils (Van Gestel et al. 1996; Mendes et al. 1999; Schutter and Dick 2002,). Ranjard and Richaume (2001) discovered that *Azospirillum irakense* inhabited only micro-aggregates; moreover, they also found that this soil fraction was a more suitable environment for bacteria in various soils.

Soil aggregates in a topical deciduous forest of western Mexico constitute around 80 % of the total mass of these soils and they play a major role in the maintenance of C storage and dynamics (Garcia-Oliva et al. 2003). Since aggregates are a key element in this forest, the purpose of this study was to analyse the bacterial community patterns among three landscape positions and two soil aggregate size fractions, and to relate these patterns with soil C and N dynamics in this tropical forest in western Mexico. We chose Terminal Restriction Fragment Length Polymorphism analysis (TRFLP) to characterize microbial communities since it overcomes most of the problems presented by other "fingerprint" approximations; i.e. resolution power, replicability, differential electrophoretic mobility, translation into taxonomic information and lack of capacity to quantify diversity (Tiedje et al. 1999; Osborn et al. 2000).

## 2. Material and Methods

*2.1 Study Area*

The study was carried out in the Chamela-Cuixmala Biosphere Reserve on the Pacific coast of Mexico (19° 30' N and 105° 01' W), as part of a research program on ecosystem functioning (Sarukhán & Maass 1990). Landscape forms are predominantly low hills with steep slopes (< 20°). The main soil parent material is rhyolite from the Tertiary period with kaolinite as the dominant clay mineral (Campo et al. 2001). Soils according to the USDA system are Typic Ustorthents (Cottler et al. 2002); about 30% of soil organic matter (SOM) (García-Oliva & Maass 1998) and 76% of the fine root productivity (Kummerow *et al.* 1990) are concentrated in the first five centimeters. The mean annual temperature and annual precipitation are 24.6 °C and 741 mm respectively (1983-2002; García-Oliva *et al.* 2002). Tropical deciduous forest (TDF) is the dominant vegetation where most of the species are leafless seven months during the year (November to May; Bullock and Solís–Magallanes 1990).

*2.2 Field sampling*

Sampling was conducted in September 2002 (the rainiest month), at three landscape positions (south-facing and north-facing mid-slopes, as well as top-hills); we chose these positions because it has been found that in these watersheds spatial and temporal variation in the Solar Radiation Index (SRI) is affected by local topographic characteristics; which in turn has a direct consequence on the evaporative demand and soil nutrient contents (López-Blanco et al 1999; Galicia et al. 1999; Zarco-Arista 2001. In each topographic position we selected seven sites according to their longitude, aspect, as well as edaphic and geo-morphological similarities (López-Blanco *et al.* 1999). In each site we established plots of 150 $m^2$ in which we randomly took composite samples of 15 undisturbed topsoil cores, from the top five centimeters, and we mixed them to obtain one composite sample per plot.

*2.3 DNA extraction and TRFLPs*

Soil samples were dry sieved into micro-aggregates (< 250 μm) and macro-aggregates (> 250 μm). We extracted the genomic DNA the same day of sampling, for both aggregate fractions, using the Ultra Clean Soil DNA Kit (Mo Bio Lab., Inc.), and stored the products at -20° C. We amplified, by PCR, the16S rRNA genes, in each sample, using fluorescently labeled domain-specific primers (Forward 515 VIC 5'GCGGATCCTCTAGACTGCAGTGCCAGCAG CCGCG GTAA-3'; Reverse 1492 6FAM 5'-GGCTCGAGCGGCCGCCCGGGTTACCTTG TTACGA CTT-3', Applied Biosystems). Three independent PCR were performed for each sample, with each PCR reaction containing 1X PCR Buffer, 1.65mM MgCl2, 0.2 mM dNTP mixture, 0.6 μM of each primer, 1 unit Taq polymerase (ABI) and 5% BSA. All reactions were carried out in a MJ research thermocycler with the following program: 94°C x 4 min; 35 cycles 92°C x 1.5 min, 50°C 1.5 min, 72°C x 2 min; 72°C x 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 Rsa, in a 20 μl reaction during 3 hours, one reaction for each enzyme (Promega). Each reaction contained 10 units of AluI (or RsaII) enzyme and 50 ng of the PCR product, digestions were run in a MJ research thermocycler with the following program, 37 °C x 3 hours and 65°C x 30 min. Size and abundance of fluorescently labeled terminal restriction fragments (t-RFs) were determined using an ABI 3100 PRISM DNA analyzer. Each t-RF was considered an operational taxonomic unit (OTU) and only those OTUs with heights ≥ 50 fluorescent units were used for the analyses.

*2.4 Bacterial communities.*

In order to compare different parameters among microbial communities we built presence and abundance matrices using OTUs with values ≥ 50 FU. These data were used to perform all the analyses described thereafter but co-ocurrence and UPGMA.

Richness. The total number of species or *species richness* is one of the simplest approaches to know the diversity and to some extent the functional activity within a community (Morin, 1999); moreover, it is an attribute that

allow us to make basic comparisons among different communities. In order to compare OTUs richness between aggregates and among sites we constructed rarefaction curves and interpolated to the smaller sample (EcoSim 6.0; Gotelli & Entsminger 2001). We also calculated the total number of OTUs and their distribution between macro and micro-aggregates and among the three sampled landscapes positions; we estimated the absolute and relative abundance of the OTUs exclusive to macro-aggregates, micro-aggregates and those shared by the two soil fractions, and we also tested for OTUs' co-occurrence between the two soil aggregate fractions, for this analysis we used presence/absence matrices (Gotelli & Entsminger, 2001)

Diversity. As it is important to know how many different species there are within a community, their abundance will give us information about the rarity, commonness or dominance of the species interacting in a given community. We measured Shannon diversity (H') and equitability (J') indexes (Magurran, 1988) with the purpose of making relative comparisons among the microbial communities associated to landscape position and associated to micro- and macro-aggregates.

UPGMA. We performed an UPGMA analysis, using presence absence matrices, to search for OTUs distribution between the soil aggregates fractions (PAUP).

*2.5 Soil Parameters Analyses*

We used the results obtained from a previous study (Chapter II, this thesis) to look for a relationship between microbial communities and soil biogeochemical parameters within the two sizes of aggregates. In order to accomplish this point we run the following tests.

"t" test. Because there were no significant differences in the biogeochemical parameters for the landscape position (Chapter II, this thesis), we run a "t" test to find any significant difference between the bigeochemical values obtained for micro- and macro-aggregates.

Pearson Correlation. We conducted a Pearson correlation test for each soil aggregate fraction in order to find out any given correlation among the biogeochemical parameters measured (total C, total N, microbial C, microbial N, labile C, $NO^{-}_{3}$, $NH^{+}_{4}$ and $CO_2$-C) within each aggregate fraction.

Stepwise regression. We carried out a stepwise regression for each soil aggregate fraction to find any relationship between the number of OTUs found and the biogeochemical variables: labile C, microbial C, microbial N, $NH_4$ and $NO_3$.

## 3. Results

### *3.1 Bacterial Communities*

We found a total of 149 different OTUs, with the highest value (55 OTUs) for the top-hill and the lowest (10 OTUs) for the south-facing mid-slopes. According to the rarefaction curves, micro-aggregates were as rich in OTUs as macro-aggregates within each topographic position. However, both size fractions were richer in the top-hills than their counterparts in the south and north-facing mid-slopes (Fig. 1). From the 149 OTUs, 25 % were only present in macro-aggregates, 25 % only in micro-aggregates, and the 50 % left was shared by the two size fractions. Those OTUs that were shared by the two aggregate sizes were also the most abundant; in contrast, those OTUs restricted to either macro or micro-aggregates were the least abundant. Additionally, the number of OTUs that co-occur in the two size particles within each position was lower than the expected by chance ($P<0.05$), that is, a non-random pattern of OTUs distribution exist between the two aggregate size-fractions. This pattern is confirmed by the UPGMA analysis in which the cluster indicates a very clear separation of OTUs between the two sizes of aggregates, independently from the position (Fig. 2). We found two big branches, one for those OTUs inhabiting macro-aggregates (green circled) and the other for those OTUs present in micro-aggregates (blue circled), with some outliers in both cases. We also found two small branches in which there is also differentiation between the two aggregate sizes.

H' values variation along the different landscape positions was almost non-existent. The values obtained ranged from 2.96 to 2.99 for macro-aggregates and from 2.66 to 2.86 for micro-aggregates. The same pattern was found for the equitability index (J') which oscillated from 0.85-0.90 and 0.78-0.89, for macro- and micro-aggregates respectively (Fig. 3).

*3.2 Soil Parameters*

According to the "t" test the average values of all biogeochemical parameters measured, but total and microbial nitrogen; were higher for micro-aggregates than for macro-aggregates ($P < 0.05$; Table 1).

Pearson correlation results showed in the case of micro-aggregates significant correlations (Table 2; $P<0.05$) between microbial C and microbial N (0.63); microbial C and $NO_3$ (0.61); $NH_4$ and $NO_3$ (-0.60); and the number of OTUs and microbial N (0.52). For macro-aggregates the correlations were positive and significant (Table 2; $P< 0.05$) between labile C and microbial C (0.75); labile C and $NO_3$ (0.78); microbial C and $NO_3$ (0.66); as well as $NH_4$ and the number of OTUs (0.49).

The stepwise regression indicated that for micro-aggregates the correlation was significant ($P < 0.05$) between the number of OTUs and microbial N ($r^2 = 0.20$); while in the case of macro-aggregates the significant correlation ($P < 0.05$) was found between the number of OTUs and $NH_4^+$ ($r^2 = 0.24$).

## 4. Discussion

Richness and diversity can give us important insights for the identification of microbial patterns of distribution and microbial functional activity in soils (Øvreås and Torsvik, 1988). We found that the top-hill was the richest topographic position, this could be explained by the specific circumstances of the watershed were the study was developed (Garcia-Oliva & Maass, 1998). According to these authors there is a differential nutrient dynamics among landscape positions:

a) Nutrient control in the top-hill is more related to biological conditions because soil stability is greater than in the slopes, and at the same time C and N inputs and accumulation of their available forms are higher in top-hills than in the slopes (Zarco, 2001). These conditions favor aggregate formation as well as nutrient enrichment, giving more opportunities to the creation of new and different niches for microorganisms. Supporting this hypothesis, Øvreås and Torsvik (1988) results indicate that soil diversity is affected by soil biogeochemical properties, and that organic soils possess more diverse microbial communities than different sandy soils as a result of the broad range of organic substrates present in the former;

b) Nutrient control in the slopes is determined by the landscape dynamics due to a continuous soil movement, which increases during the rainy season, specially with cyclones (García-Oliva, et al. 1995). This creates a micro-environment that is changing continuously, producing the lost of organic matter and nutrients as they are incorporated (Garcia-Oliva & Maass 1998; Zarco-Arista 2001).

Equitability indexes (J') were very similar among the communities studied and the values found were very close to one, indicating that no particular OUT predominated within the different communities. Zhou *et al.* (2002) proposed different mechanisms that can turn out into a non-competitive pattern within a community: "super abundant resources, resource heterogeneity, spatial isolation and, non-equilibrium conditions". In this particular case we can attribute the high diversity and equitability to two of the mechanisms mentioned: super-abundant resources and resource heterogeneity:

a) *Super-abundant resources.* TDF is characterized by a period during the year with a high availability of resources (i.e. water and nutrients) at the onset and during the rainy season. The soil is enriched, at the onset of the rainy season, with the nutrients leached from the litter surface, starting with the mineralization process that will sustain vegetation as well as microorganisms in the soil (Singh et al. 1989; Campo et al. 1998; García-Oliva

et al. 2003). Even though during the rainy season C and N availability is lower than in the previous seasons (dry and beginning of rains), concentrations of these nutrients are higher than in temperate, desert or agricultural systems, allowing high diverse an non-competitive communities and;

b) *Resource heterogeneity*. A variety of data, including our results, demonstrate the existence of differential nutrient characteristics within macro- and micro-aggregates, giving microorganisms the possibility to utilize diverse "food" resources (Ranjard and Richaume 2001).

Diversity (H') and equitability (J') values were very similar for both sizes of aggregates and the different landscape positions, possibly because the most common OTUs were also the most abundant. Nevertheless, it is important to emphasize the existence of a non-random pattern of distribution between micro- and macro-aggregates. The fact that only 50% of the total number of OTUs is shared by both fractions and that there is a group of OTUs that are only present either in micro or macro-aggregates, with low probability of co-ocurrence, give us some insights about the distinct functional roles that these specific and exclusive OTUs may play in each fraction. This point was more obvious when we analyzed the effect of aggregate size-fraction on biogeochemical processes.

We found that micro-aggregates presented higher values for all the parameters measured, with exception of total and microbial N, the values *per se* indicate a differential storage capacity and resource use between the two aggregate sizes. By September (rainy season) these had a considerable lost of nutrients, mainly total N which is lost more rapidly than total C. The C/N ratios confirm that a distinct C dynamics exist, and that processes are apparently more stable in macro- than in micro-aggregates (Chapter II, this thesis). Similarly, the availability of C is also reduced (Garcia-Oliva et al 2003; Chapter II, this thesis), constraining the activity of heterotrophic populations and enhancing nitrification (Vitousek et al.1982, Hart et al. 1994).

It is interesting to mention that the number of OTUs for micro-and macro-aggregates are related to different N forms (microbial N and $NH_4^+$,

respectively), suggesting that the amount of OTUs are directly related to N transformations. In addition, the clear OTUs differentiation between the two aggregate size fractions indicates that those microorganisms that are exclusive, either of macro-aggregates or micro-aggregates, are accomplishing distinct biogeochemical processes related to nitrogen dynamics, even though their abundances are much lower than that of the microorganisms shared between the two aggregate fractions.

Moreover, the number of OTUs in micro-aggregates is explained (Pearson correlation and Stepwise regression) by microbial nitrogen, which indicates that in this soil size-fraction there is greater heterotrophic microbial species than in macro-aggregates, enhancing N immobilization. The positive correlation between microbial C and microbial N and the negative correlation between $NH_4^+$ and $NO_3^-$ also suggest an important activity of the heterotrophic populations and a greater C mineralization within micro-aggregates. Apparently, bacteria are more protected within the micro-aggregate fraction of the soil, as mentioned in other studies (Hattori 1998; Foster and Dormaar 1991), giving as a result a redistribution of the microbial N forms in a time of the year where N lost and N plant absorption are dominant (Singh et al. 1989, Roy and Singh 1995).

In contrast, within macro-aggregates the number of OTUs correlated with $NH_4^+$, which could be an indication that microorganisms rely on $NH_4^+$ availability for N immobilization ( in the case of heterotrophs) or nitrification. It also seems that nitrification is higher in macro-aggregates than in micro-aggregates as suggested by the $NH_4^+/NO_3^-$ ratio (0.16 and 0.22 respectively), indicating protection of the heterotrophic population by micro-aggregates during the rainiest months.

Our results indicate that the microbial communities found in the two soil aggregate size-fractions have different composition and that those organisms inhabiting either macro- or micro-aggregates are more closely related. Furthermore, we can assume that the least abundant microorganism are not necessarily the least important; on the contrary, our data show that the least

abundant microorganisms are the ones playing the different biogeochemical processes within each size fraction, and also that they are carrying out these processes at different rates.

Acknowledgments

The authors thank Laura Espinosa, Maria Schneider, Stephen Bent and May Wong for their technical assistance in the molecular analyses; Salvador Araiza, Carlos Anaya and Manuel Montaño for their assistance during the field work; Leopoldo Valiente for his comments on the statistical analyses; Heberto Ferreira for his assistance in processing data; and the personnel of the Chamela Biological Station, *UNAM*, for providing us with the logistic support during the field work. This project was financed by *CONACyT*, México (Grant No. G27674-N) and by NASA (Grant No. NC2-1051).

Fig. 1 Rarefaction curves for macro- (Ma) and micro-aggregates (Mi) in the three different landscape positions (TH=top-hill; SS=south-slope; NS=north-slope).

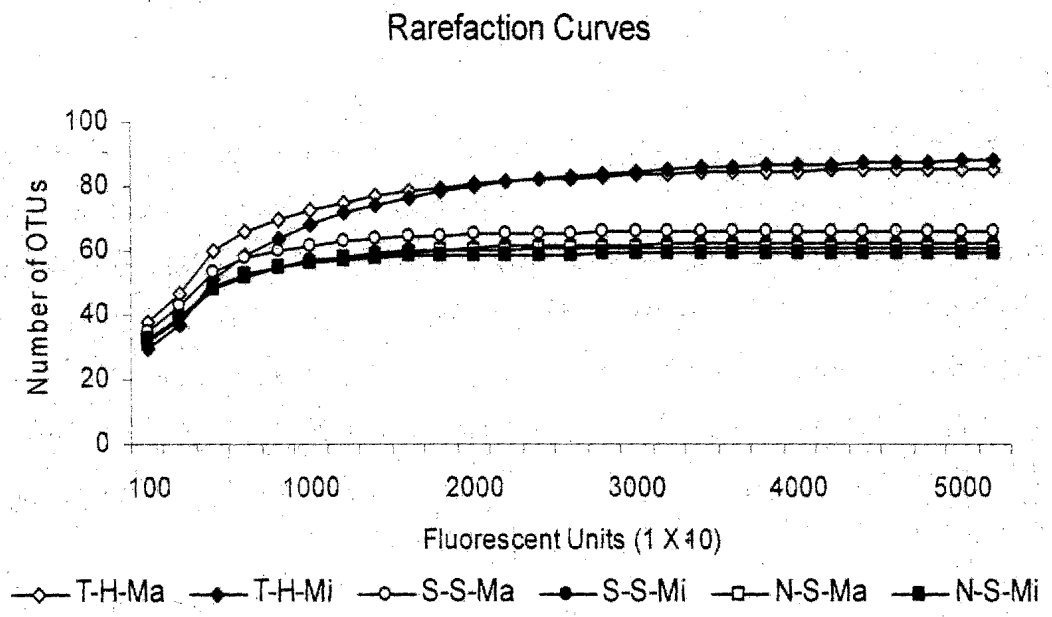

Figure 2. UPGMA analysis for the OTUs found in soil macro- and micro-aggregates.

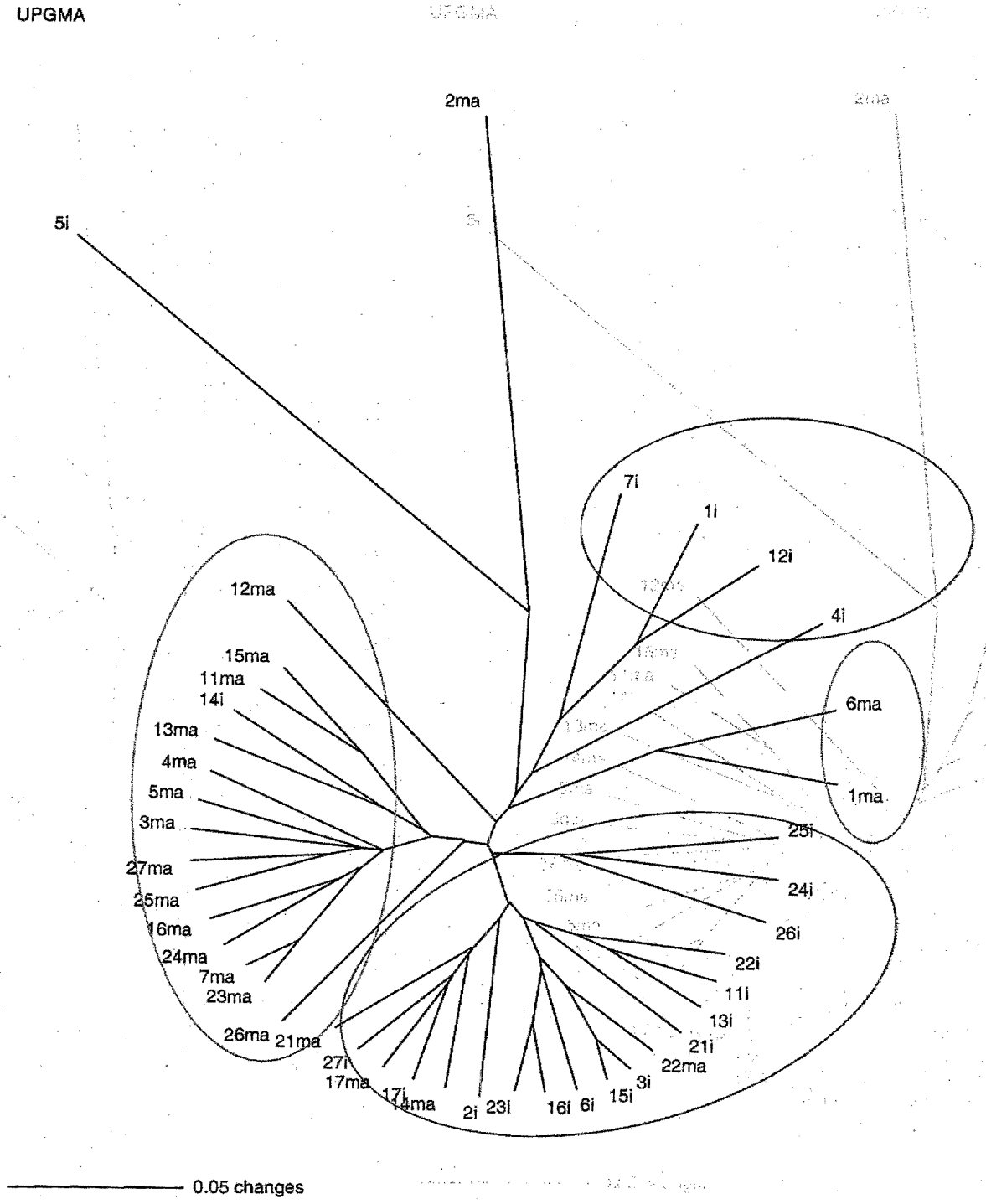

Figure 3. Shannon diversity (H) and equitability (E) indexes for the two aggregate fractions in the three different positions studied, Ma (macro-aggregates), Mi (micro-aggregates).

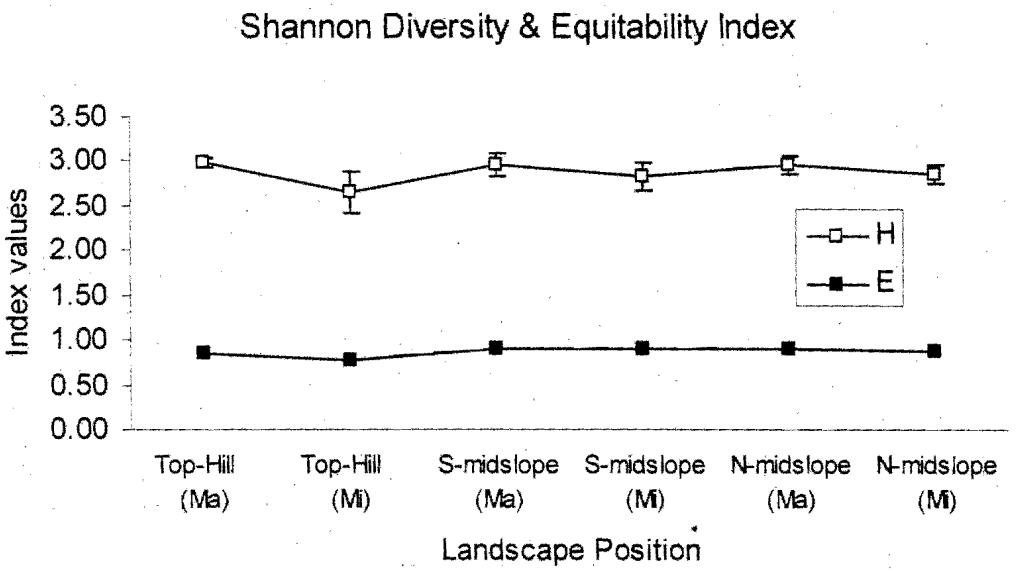

Table 1. Mean values and "t-test" probabilities for total, available and microbial nutrients in macro- and micro-aggregates for the rainy season period (September).

| Nutrients | Macro-aggregates | Micro-aggregates | (P) |
|---|---|---|---|
| Totals ($mgg^{-1}$) | | | |
| Carbon | 27(3.6)$^{b}$ | 40(5.1)$^{a}$ | 0.000* |
| Nitrogen | 2.8(0.4)$^{a}$ | 3.3(0.3)$^{a}$ | 0.076$^{ns}$ |
| C/N ratio | 10(0.7)$^{b}$ | 13(2.3)$^{a}$ | 0.038* |
| Available ($\mu gg^{-1}$) | | | |
| $C_{labile}$ | 99.7(25)$^{b}$ | 160(23)$^{a}$ | 0.015* |
| $NO_3$ | 89(10)$^{b}$ | 112(14)$^{a}$ | 0.000* |
| $NH_4^+$ | 13.5(2.3)$^{b}$ | 24.7(4.7)$^{a}$ | 0.000* |
| Microbial ($mgg^{-1}$) | | | |
| Carbon | 617(95)$^{b}$ | 820(125)$^{a}$ | 0.000* |
| Nitrogen | 12.2(1.9)$^{a}$ | 13.9(4.6)$^{a}$ | 0.385$^{ns}$ |
| Mineralization ($\mu gg^{-1}d^{-1}$) | | | |
| $CO_2$-C | 12(1.4)$^{b}$ | 20(3.5)$^{a}$ | 0.001* |

Table 2. Pearson correlations among biogeochemical parameters for (*a*) macro-aggregates and (*b*) micro-aggregates.

*(a)*

| Macro-aggregates | $C_{labile}$ | $C_{microbial}$ | $CO_2$ production | $NH_4$ | $N_{microbial}$ | $NO_3^-$ |
|---|---|---|---|---|---|---|
| $C_{microbial}$ | 0.751 | | | | | |
| P-value | 0.000* | | | | | |
| $CO_{2(production)}$ | -0.276 | -0.151 | | | | |
| | 0.252 | 0.536 | | | | |
| $NH_4$ | -0.425 | -0.275 | 0.115 | | | |
| | 0.0695 | 0.252 | 0.638 | | | |
| $N_{microbial}$ | 0.208 | 0.384 | -0.307 | -0.398 | | |
| | 0.391 | 0.109 | 0.200 | 0.091 | | |
| $NO_3$ | 0.797 | 0.663 | -0.189 | -0.331 | 0.083 | |
| | 0.000* | 0.001* | 0.436 | 0.165 | 0.734 | |
| OTUs | 0.0398 | 0.132 | 0.037 | 0.0485 | -0.376 | -0.0201 |
| | 0.871 | 0.589 | 0.879 | 0.035* | 0.111 | 0.407 |

*(b)*

| Micro-aggregates | $C_{labile}$ | $C_{microbial}$ | $CO_2$ production | $NH_4$ | $N_{microbial}$ | $NO_3^-$ |
|---|---|---|---|---|---|---|
| $C_{microbial}$ | -0.277 | | | | | |
| P-value | 0.281 | | | | | |
| $CO_{2(production)}$ | -0.144 | 0.400 | | | | |
| | 0.580 | 0.11 | | | | |
| $NH_4$ | 0.026 | -0.239 | -0.180 | | | |
| | 0.921 | 0.355 | 0.488 | | | |
| $N_{microbial}$ | 0.035 | 0.634 | 0.077 | -0.302 | | |
| | 0.893 | 0.006* | 0.766 | 0.238 | | |
| $NO_3$ | -0.329 | 0.617 | 0.198 | -0.598 | 0.278 | |
| | 0.196 | 0.008* | 0.445 | 0.011* | 0.279 | |
| OTUs | -0.129 | 0.138 | 0.135 | 0.121 | 0.514 | -0.174 |
| | 0.620 | 0.597 | 0.603 | 0.642 | 0.034* | 0.503 |

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# CAPÍTULO IV

# MICROBIAL MACROECOLOGY: HIGHLY STRUCTURED PROKARYOTIC SOIL ASSEMBLAGES IN A TROPICAL DECIDUOUS FOREST

biogeographical barriers (Finlay, 2002) than microbial eukaryotes. Scant empirical evidence suggests that this generalization might be true for oceanic bacteria, but not for soil or sediment prokaryotic assemblages (Finlay & Clark, 1999; Finlay *et al.*, 1999; Torsvik *et al.*, 2002; Nee, 2003; Grundmann, 2004). However, no study has performed a sampling procedure designed specifically to address this issue, and the question of whether bacteria show biogeography or not remains unanswered (Curtis *et al.*, 2002; Nunan *et al.*, 2002; Fenchel, 2003).

We define a syndrome of ubiquity for species 'with no biogeography' to include the following traits: (1) a high local to global species ratio, meaning that a single site can contain a high percentage of the full global species set, which is comparatively small; (2) a very high dispersal rate, coupled with a very high abundance of individuals, providing a huge 'seedbank' of species; (3) extremely large distributional ranges, with very few or no species with restricted distribution; (4) a very low rate of species turnover (beta diversity), so samples tend to contain the same species regardless of the physical distance between them; (5) a flat species–area curve; and (6) unstructured local communities, which are random subsamples of the global species pool.

Available information seems to show that not all prokaryotes are cosmopolitan, and that at least some species do not show traits 1 and 2 of the syndrome of ubiquity (Massana *et al.*, 2000; Curtis *et al.*, 2002; Nunan *et al.*, 2002; Torsvik *et al.*, 2002; Fenchel, 2003; Whitaker *et al.*, 2003). Studies on the distribution of guild members, phylogenetically related populations (Cho & Tiedje, 2000) and particular species (Whitaker *et al.*, 2003) are consistent with the conclusion that prokaryotic species can be restricted to given locations, and their distribution probably reflects adaptive evolution to local conditions. In contrast, pathogenic bacterial species and *Bacillus* spore formers are reported to have global panmictic distributions (Massana *et al.*, 2000). Similarly, studies on other free-living prokaryotes have found apparently identical microorganisms in equivalent, but geographically separated environments, such as polar oceans (Hollibaugh *et al.*, 2002), ice (Staley & Gosink, 1999) and marine sediments (Bowman & McCuaig, 2003). Unfortunately, assertions concerning the biogeography of prokaryotes are largely based on fragmentary information, and the pattern of beta diversity, or how similar in species composition are the samples taken from different places, has not been examined (Nee, 2003). Also unexplored is the pattern in which the count of prokaryote species varies with the sampling scale (Grundmann, 2004). Knowing the pattern of beta diversity at different scales, researchers can make inferences regarding the distributional ranges of species, the species–area relationship, and the degree of randomness of local communities (Godfray & Lawton, 2001; Whittaker *et al.*, 2001; Arita & Rodríguez, 2002; Ricklefs, 2004). Here, we use such relationships to test the hypothesis that prokaryote assemblages show traits 3 to 6 of the syndrome of ubiquity.

## MATERIALS AND METHODS

We examined the composition of prokaryotic soil assemblages at four spatial scales by systematically sampling sites within a fully nested system of quadrats (Fig. 1; Arita & Rodríguez, 2002). This sampling design allowed us to measure distribution, taxonomic diversity (see definition of our operational taxonomic units below) and beta diversity at four spatial scales ($A_0$, $A_1$, $A_2$, $A_3$). Starting with a quadrat of side $L_0 = 8$ m (and area $A_0 = 64$ m$^2$), containing $S_0$ taxa, we divided the sampling area into four smaller quadrats of side $L_1 = L_0/2 = 4$ m, area $A_1 = A_0/4 = 16$ m$^2$, and containing an average of $S_1$ taxa. By iterating the subdivision, we completed a series of increasingly smaller quadrats of side $L_2 = 2$ m and $L_3 = 1$ m, and area $A_2 = 4$ m$^2$ and $A_3 = 1$ m$^2$, containing $S_2$ and $S_3$ taxa, respectively. We used a checkerboard sampling design, including 32 of the 64 possible quadrats of size $A_3$, to optimize available resources without compromising the analytical power (Fig. 1). Using such design, we had at least two replicates for all samples at all scales, and this assured us against possible technical failures or sample losses. In fact, two of our samples yielded no DNA, but the robustness of the design allowed us to perform the comparisons without any loss of analytical power.

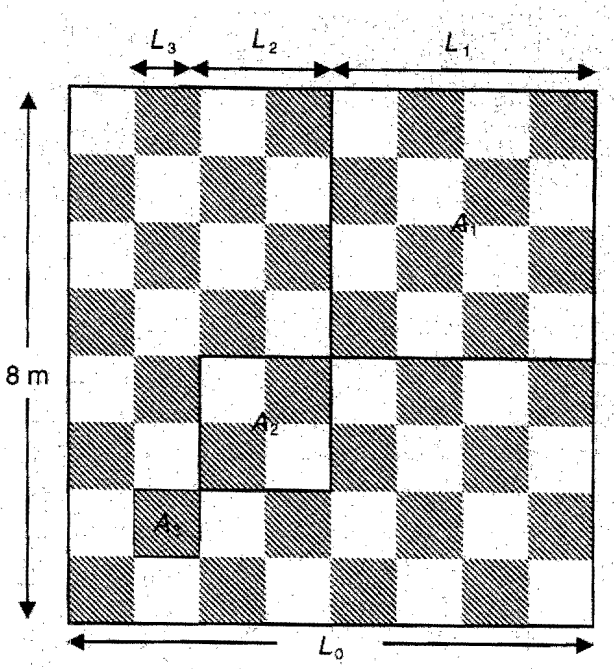


**Figure 1** Fully nested system of quadrats designed to analyse the scaling of species diversity. An 8 × 8 m quadrat of area $A_0 = 64$ m$^2$ is divided into four quadrats of area $A_1 = 16$ m$^2$, 16 quadrats of area $A_2 = 4$ m$^2$, and 64 quadrats of area $A_3 = 1$ m$^2$. For clarity, only one quadrat of each size is marked. A soil sample was taken inside 32 of the smallest quadrats, following a checkerboard pattern. Operational taxonomic unit (OTU) diversity was measured at the four scales: $S_0$ is the total diversity found in the large quadrat; $S_1$, average cumulative diversity in the four quadrats of area $A_1$ (each including eight soil samples); $S_2$ is the average cumulative diversity in the 16 quadrats of area $A_2$ (each including two soil samples); and $S_3$ is the average diversity in the 32 sampling units.

This sampling scheme was deployed at two locations of the Chamela-Cuixmala Biosphere Reserve, on the western coast of Mexico (19°30′ N, 105°05′ W). One location was a flat hilltop,

Global Ecology and Biogeography, (Global Ecol. Biogeogr.) (2005) 14, 241–248

RESEARCH PAPER

# Microbial macroecology: highly structured prokaryotic soil assemblages in a tropical deciduous forest

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## ABSTRACT

**Aim** To assess the hypothesis that free-living prokaryotes show a pattern of 'no biogeography' by examining the scaling of soil prokaryotic diversity and by comparing it with other groups' biogeographical patterns.

**Location** Two sites in the tropical deciduous forest of Chamela, Jalisco, on the western coast of Mexico.

**Methods** We examined the diversity and distribution of soil prokaryotes in two $8 \times 8$ m quadrats divided in such manner that we could sample at four spatial scales. Restriction fragment length polymorphisms of *16S rRNA* genes were used to define operational taxonomic units (OTUs) that we used in lieu of species to assess diversity.

**Results** We found highly structured species assemblages that allowed us to reject multiple predictions of the hypothesis that soil bacteria show 'no biogeography'. The frequency distribution of range size (measured as the occupancy of quadrats) of OTUs followed a hollow curve similar to that of vertebrates on continents. Assemblages showed high levels of beta diversity and a non-random nested pattern of diversity. OTU diversity scaled with area followed a power function with slopes $z = 0.42$ and 0.47.

**Main conclusions** We demonstrate a non-ubiquitous dispersal for soil prokaryotes, which suggests a complex biogeography similar to that found for terrestrial vertebrates.

**Keywords**
Bacterial diversity, beta diversity, biogeography, distribution, scales, soil prokaryotes, TRFLP.

## INTRODUCTION

Prokaryotic species are essential components of the biosphere because they catalyse processes that are critical to sustaining life on Earth. In recent years, methods based on the phylogenetic analyses of the small subunit ribosomal RNA gene sequences have expanded dramatically our understanding of prokaryotic diversity (Hugenholtz *et al.*, 1998; Curtis *et al.*, 2002). Nevertheless, only 26 of the more than 50 major lineages (Phyla) of the domain *Bacteria* are represented in cultivated strains (Rappé & Giovannoni, 2003), and there are only about 4500 species that have been characterized. Considering that more than half a million bacterial species could occur in 30 g of soil, according to some estimates (Dukhuizen, 1998), it is clear that most of the diversity of prokaryotes remains unexplored.

A direct consequence of the insufficient knowledge on the diversity of prokaryotes is an almost total lack of information regarding their distribution and biogeography. A current debate is on whether microbial communities show patterns of distribution and diversity similar to those of macroscopic organisms (Godfray & Lawton, 2001; Finlay, 2002; Nee, 2003; Horner-Devine *et al.*, 2004). Recent research shows that free-living microbial eukaryotes (e.g. protozoa and microalgae) are cosmopolitan, so the same species are found in sites in any part of the world, implying a very low rate of species turnover (beta diversity) and a low global species diversity (Finlay & Clark, 1999; Finlay *et al.*, 1999; but see Foissner, 1999). This pattern of 'no biogeography', meaning a global homogeneous distribution, has been assumed to hold also for prokaryotes, arguing that their smaller size and higher abundance make them even less prone to be bounded by

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and the second was a south-facing mid-slope (27°) of a small watershed that has been extensively studied for a long-term project on ecosystem function. Distance between the two locations was 300 m. Mean annual temperature is 24.9 °C and the mean annual precipitation is 763 mm, with the rainfall concentrated in a clearly marked wet season that lasts from June to October, showing a peak in September (García-Oliva *et al.*, 1991). The dominant vegetation is tropical deciduous forest, where most tree species are leafless during the dry season. Soils are sandy clay loams (Orthents in the United States Department of Agriculture [USDA] classification), poorly developed, with an organic matter content of < 5%, mainly concentrated in the top 5 cm, and with a pH of 6.9 (García-Oliva *et al.*, 2003).

On June 25, 2002, following the checkerboard sampling design (Fig. 1), we collected 5-$cm^3$ core soil samples from 32 of the 64 quadrats of size $A_3$ in each location, sieved them to remove gravel and other large (> 2 mm) material, and extracted genomic DNA from a 1-g aliquot of each sample. We assessed the diversity of prokaryotes based on restriction fragment length polymorphisms (RFLP) of *16S rRNA* genes that were used to define operational taxonomic units (OTUs). Genomic DNA extraction was performed on the same day of sampling from an aliquot of 1 g of sieved soil using the Ultra Clean Soil DNA kit (Mo Bio Laboratory, Inc.) and the products were stored at –20 °C. The *16S rRNA* genes in each sample were PCR(polymerase chain reaction)-amplified using fluorescently labelled domain-specific primers (forward 515 VIC 5′GCGGATCCTCTAGACTGCAGTGCCAG-CAG CCGCG GTAA-3′; reverse 1492 6FAM 5′-GGCTCGAGCG-GCCGCCCGGGTTACCTTG TTACGA CTT-3′, Applied Biosystems; Angert *et al.*, 1998). These are universal primers that target prokaryotic genes, so our results can be generalized to all groups of both Archaea and Eubacteria.

Three independent PCRs were performed for each sample, with each PCR containing 1X PCR buffer, 1.65 mM $MgCl_2$, 0.2 mM dNTP mixture, 0.6 μM of each primer, 1 unit *Taq* polymerase (ABI) and 5% BSA. All reactions were carried out in an MJ research thermocycler with the following program: 94 °C × 4 min; 35 cycles at 92 °C × 1.5 min, 50 °C 1.5 min, 72 °C × 2 min; and 72 °C × 10 min. To minimize PCR biases because of preferential amplification and reannealing, we standardized and set the optimum PCR conditions for our environmental samples as suggested by Osborn *et al.* (2000). We used the same DNA concentration and chose the number of cycles and the annealing temperature in order to obtain the best product, without compromising PCR quality. We also performed tests with different *Taq* polymerases until finding the most appropriate for our case. Tillmann and Friedrich (2003) found that there are no significant differences in terminal restriction fragment-length polymorphism (TRFLP) obtained between 28 and 45 PCR cycles and that temperature annealing should be set for the particular primer.

PCR products were combined and purified from a 2% agarose gel (Gel extraction kit Qiagen, Inc.). The amplicons were restricted using *Alu*I enzyme (Promega) in a 20 μL reaction during 3 h. Each reaction contained 10 units of *Alu*I enzyme and 50 ng of the PCR product, digestions were run in an MJ research thermocycler with the following program: 37 °C × 3 h and 65 °C × 30 min. Size and abundance of fluorescently labelled terminal restriction fragments (t-RFs) were determined using an ABI 3100 PRISM DNA analyser.

Each t-RF was considered an OTU and only those with heights of ≥ 50 fluorescent units (FU) were used for the analysis. Thresholds are chosen by assessing the noise in a region known to have no fragments, based on the particular background noise produced for each machine and on the appearance of peaks in samples run only with a control. Studies have shown that by cutting peaks at 100 FU or greater, there is an increase in the number of errors found (Blackwood *et al.*, 2003).

Characterization of microbial communities has been hindered in the past by traditional culture methods, because only a very small fraction of microorganisms found in environmental samples could be recovered. Recently, several molecular techniques have been developed to study phylogenetic relationships and diversity in microorganisms (Liu *et al.*, 1997; Tiedje *et al.*, 1999; Ranjard *et al.*, 2000; Norris *et al.*, 2002; Hill *et al.*, 2003). Among these, TRFLPs overcome most of the problems plaguing other fingerprinting approaches in terms of low resolution power, lack of replicability, differential electrophoretic mobility, and lack of capacity to quantify diversity. In particular, TRFLPs are very useful in comparing different communities because of their high level of sensitivity and replicability (Blackwood *et al.*, 2003).

For each location, we constructed presence–absence matrices describing the distribution of OTUs among 30 quadrats of size $A_3$ in the hilltop and 32 quadrats in the slope (we were unable to extract usable DNA from two of the hilltop samples). The purpose of the sampling procedure was not to measure the total OTU diversity of sites, a goal that is not feasible for prokaryotes with existing methods. Instead, the objective was to assess the spatial patterns of diversity by conducting a standardized sampling procedure that allowed us to carry out valid comparisons among quadrats. Thus, we assessed the adequacy of the sampling by its statistical representativeness (Gilbert, 1987) and not by a criterion of completeness, as in inventory-orientated studies (Gotelli & Colwell, 2001).

In environmental studies, a parameter is considered adequately sampled if the probability of a 20% variation around the mean value is < 0.1 (Gilbert, 1987). The probability can be estimated with the formula $Z_{1-\alpha/2} = \sqrt{n}d_r/\eta$, where $\alpha$ is the probability, $Z_{1-\alpha/2}$ is the value for the standardized normal distribution, $n$ is the number of samples, $d_r$ is the chosen acceptable relative error ($d_r = |\bar{x} - \mu|/\mu$; where $\bar{x}$ is the measured average and $\mu$ is the true, unknown population mean), and $\eta = \sigma/\mu$ (where $\sigma$ is the true population standard deviation). Using this formula, we assessed the adequacy of our measurement of diversity at scale $A_3$, estimating $\sigma$ with the observed standard deviation ($s$) and $\mu$ with the observed sampling mean ($\bar{x}$).

Rarefaction curves were built for the two sites by plotting the cumulative number of OTUs as a function of increasing numbers of samples. We used EstimateS version 7.0 (Colwell, 2004) to calculate the points of our rarefaction curves, using the procedures of Colwell *et al.* (2004) that allow the exact calculation of expected diversity values and associated variances for any number of samples (see also Ugland *et al.*, 2003).

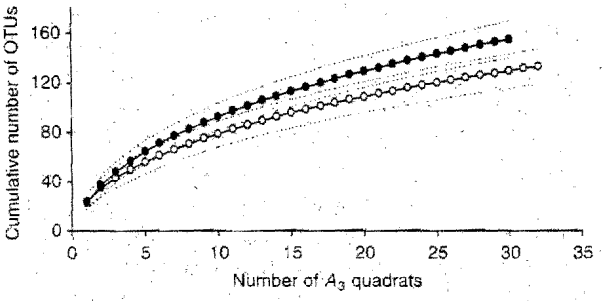


**Figure 2** Rarefaction curves of OTU diversity for 30 soil samples in a hilltop (filled markers) and 32 samples in a slope (empty markers) in a tropical dry forest of western Mexico. Broken lines show the 95% confidence intervals for the means. The curves were built using the exact solution of Colwell *et al.* (in press) as implemented in EstimateS version 7.0.

We assessed β diversity at three scales using Whittaker's (1972) formulation $\beta_i = S_{i-1}/S_i$, where $S_i$ is the average species diversity in quadrats of area $A_i$ (Arita & Rodríguez, 2002). To determine the shape of the OTU–area relationship [equivalent to the species-area relationship (SAR)], we performed non-linear regressions of average OTU diversity against area for the two sampling sites. We examined the structure in the assemblages by measuring their degree of nestedness. In a perfectly nested assemblage, OTUs found in poor sites occur also in more diverse sites. Nestedness is a correlate of order or structure within communities, and can be measured with a temperature value (Atmar & Patterson, 1993). Low temperatures are characteristic of highly nested assemblages showing low degrees of disorder. The significance of the nestedness measure was assessed by assembling 1000 random sets of species using the temperature calculator of Atmar and Patterson (1995).

## RESULTS AND DISCUSSION

### OTU diversity

We documented the presence of 198 OTUs in the two sites. Of these, 155 occurred in the 30 samples from the hilltop and 133 in the 32 samples from the slope, with 56 (36.1%) and 34 (25.6%) taxa exclusively found in the hilltop and the slope, respectively. Thus, overall, only 108 of the 198 identified OTUs (54.5%) occurred in both sites. The hilltop was richer in OTUs than the slope, even after taking into account the differing sample sizes, as shown by rarefaction curves (Fig. 2).

Full inventories of prokaryotic taxa are not feasible with currently available techniques. To analyse patterns of diversity for this group, as for other highly diverse organisms, such as beetles, tropical butterflies or aquatic invertebrates, researchers rely on sampling to generate diversity estimates at different spatial or temporal scales (Gotelli & Colwell, 2001). Those estimates are comparable only if standardized field techniques are employed and if provisions are taken to consider the effect of differing sampling effort. The purpose of our study was not to measure the total prokaryotic diversity of sites, but to analyse spatial patterns in the distribution of diversity by comparing quadrats in which standard sampling and analytical procedures were performed. Because of the nature of our molecular techniques, we concentrated on the numerically dominant organisms, those with higher probabilities of being detected by our DNA analysis. Our molecular threshold (50 FU) established the 'veil line' (Preston, 1962a,b) that separated the detectable from the non-detectable taxa, in the same manner that sampling effort marks the veil line in diversity studies for other groups, such as moths and beetles. In those cases, valid comparisons can be made if standard field, laboratory, and statistical procedures are followed for all samples.

In assessing the representativeness of the sampling procedure, we found that the probabilities of sustaining a relative error of 20% or larger ($d \geq 0.2$) in measuring $S_3$ with $n = 30$ samples for the hilltop and $n = 32$ samples for the slope were $P = 0.064$ and $P = 0.0002$, respectively (in both cases, $P < 0.1$). Thus, the amount of variance (and thus, of potential bias) of our measures of $S_3$ OTU diversity at the two sites is low enough to make valid comparisons. Because of the fully nested design, estimations of $S_0$, $S_1$, and $S_2$ diversities, which are based on combinations of $S_3$ diversities put on a spatially explicit design, are also adequately sampled.

### Occupancy

Within sites, the frequency distribution of occupancy of OTUs (occupancy defined as the number of quadrats in which a given OTU is present) followed a unimodal, right-skewed ('hollow') curve, which is the most common shape for a variety of organisms, from foraminifers to trees and vertebrates (McGeoch & Gaston, 2002). The curve is also very similar to that of the frequency distribution of range size for vertebrates in continental masses (Brown *et al.*, 1996; Gaston, 2003). However, the frequency distribution for occupancy differed from a log-normal distribution (test for normality using log-transformed data, $P < 0.001$), showing an overrepresentation of OTUs that occurred in very few quadrats. Sixty-eight (44%) of the 155 OTUs recorded in the hilltop and 56 (42%) of the 133 OTUs in the slope were detected in only one A3 sample. In contrast, only two OTUs in the hilltop and seven in the slope were detected in more than 25 samples in each location (Fig. 3a). There was a significant correlation between the occupancy in the two sites, that is, OTUs that were widespread in the hilltop were also widespread in the slope ($r = 0.875$, calculated as $n = 198$ occupancy pairs, $P < 0.001$).

In any study of species distribution, there is the potential problem of bogus patterns emerging from incomplete sampling. It is possible that the occurrence of some OTUs in some quadrats might have gone undetected because of our chosen molecular threshold. However, the effect of this potential problem, which is common to all studies based on sampling, is likely to be of minor importance. If we could lower the threshold to an imaginary level that allowed us to have a complete inventory of OTUs, it is likely that some of the OTUs would be detected in more quadrats than presently reported (that is, some OTUs would have a larger occupancy). However, by lowering the threshold, we would also

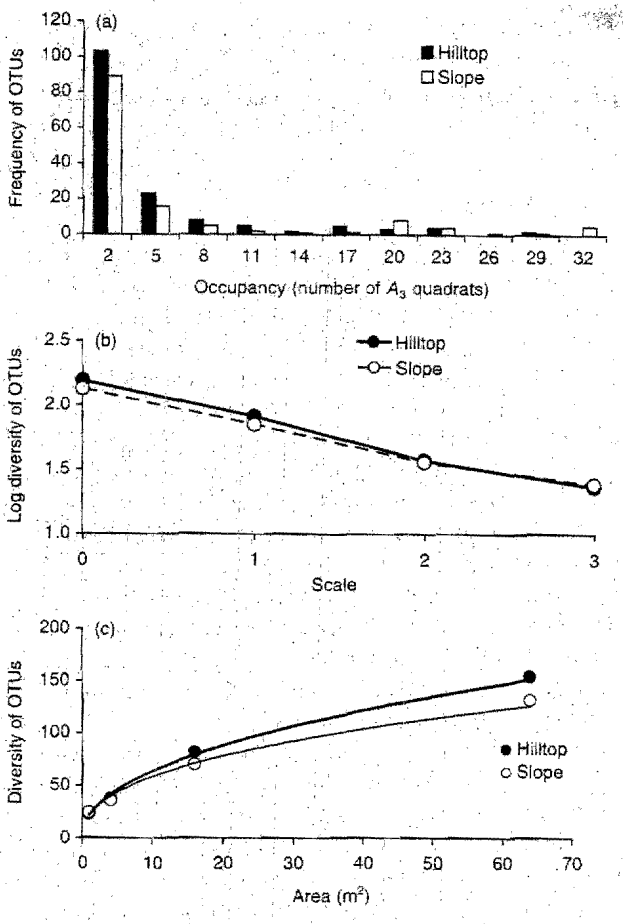


**Figure 3** Diversity patterns of soil prokaryotes in two locations of a tropical dry forest in western Mexico. Black marks indicate hilltop samples and white mid-slope samples. (a) Frequency distribution of occupancy (number of occupied sampling quadrats) for prokaryotic OTUs in the two 64-$m^2$ squares. (b) OTU diversity-scale plots for the two 64-$m^2$ squares, showing OTU diversity as a function of scale as explained in Figure 1. (c) Species-area curve; data for OTUs from the two 64-$m^2$ squares were pooled to calculate the regression; the rightmost point is the total cumulative diversity in the two squares.

be able to detect many more of the rarest OTUs, those occurring at extremely low densities and, most likely, in fewer quadrats. We contend that by lowering the threshold, or by performing a more intense sampling, we would simply move Preston's (1962a,b) veil line, but that the shape of the histograms shown in Fig. 3(a) would not change significantly.

Thus, by documenting the presence of OTUs with extremely restricted distribution and by demonstrating that a large percentage of OTUs are found in only one of two locations, we rejected prediction 3 of the syndrome of species with no biogeography.

## Scaling and OTU–area relationship

Assemblages of prokaryotic taxa followed similar scaling trends in both locations, as shown by the OTU scale plots (Fig. 3b). In these plots, log species diversity or log OTU diversity are functions of spatial scale, in this case, scales 0, 1, 2 and 3 corresponding to areas $A_0 = 64$ m$^2$, $A_1 = 16$ m$^2$, $A_2 = 4$ m$^2$, and $A_3 = 1$ m$^2$, respectively. The slope of the regression line is equal to $-\log \beta$, where $\beta$ is Whittaker's (1972) beta diversity (Arita & Rodríguez, 2002). When $\beta$ is small, there is very little species turnover and samples contain about the same OTUs regardless of their size; whereas large $\beta$ values mean a high turnover rate that implies marked differences in the composition of OTUs among samples. Regression analysis for both of our locations fit a straight line with slopes –0.281 ($\beta = 1.91$, $r^2 = 0.99$) for the hilltop and –0.251 ($\beta = 1.78$, $r^2 = 0.99$) for the slope. Note that only the average values for each scale were used to perform the regressions to avoid pseudoreplication and reduce the effect of spatial autocorrelation. These results indicate that an increment in quadrat area by a factor of four represents an increase in the diversity of OTUs by a factor of $\beta = 1.91$ in the hilltop and by $\beta = 1.78$ in the slope. Arita and Rodríguez (2002) used the same sampling procedure as ours but with $A_0$ quadrats of 180,000 km$^2$ and found that $\beta$ diversity for non-volant Mexican mammals ranged from 1.19 in a homogeneous (the Yucatán Peninsula) to 2.52 in a highly heterogeneous area (central México). Figures for $\beta$ diversity of prokaryotes in our 64-$m^2$ locations correspond to high-end values for mammals in quadrats that are approximately $2.8 \times 10^9$ times larger in area. Hence, prediction 4 of the syndrome of ubiquity, a low rate of species turnover, can be unequivocally rejected for prokaryotes in our locations.

Linear OTU scale plots imply OTU–area relationships of the form $S = cA^z$, where $c$ and $z$ are constants (Rosenzweig, 1995; Harte *et al.*, 1999; Arita & Rodríguez, 2002). Performing nonlinear regressions, we estimated $z = 0.47$ for the hilltop and $z = 0.42$ for the slope ($r^2 = 0.98$ for both cases, Fig. 3c). These $z$ values are higher than reported values for vertebrates in nested sampling units in continents (Rosenzweig, 1995), and are much higher than for invertebrates in the sea ($z = 0.161$, Azovski, 2002) and for ciliated protists ($z = 0.043$, Finlay, 2002). Prokaryotes in our locations clearly do not show a flat species–area curve; therefore prediction 5 of the syndrome of ubiquity can be safely rejected.

Our sampling design (quadrats arrayed in a contiguous grid) yielded type II OTU–area curves in the classification of Scheiner for species–area relationships (2003, 2004). A related sampling procedure uses strictly nested quadrats (type I in Scheiner, 2003), in which only one quadrat is sampled at each scale and smaller quadrats are nested within larger ones. The theoretical implications of such design has been explored by Harte *et al.* (1999), and similar sampling designs has been used for the analysis of the continental distribution of species diversity (e.g. Lyons & Willig, 2002). The design suffers, in our view, from the lack of replicates and from the fact that smaller scales cover only limited parts of the whole region, going to the extreme, where the smallest scale is represented by a single point (at the centre or at one extreme of the region). The sampling design used herein, in contrast, systematically arrays quadrats of every scale covering the whole region, providing true replicates and a better depiction of the spatial variation of diversity (Arita & Rodríguez, 2002).

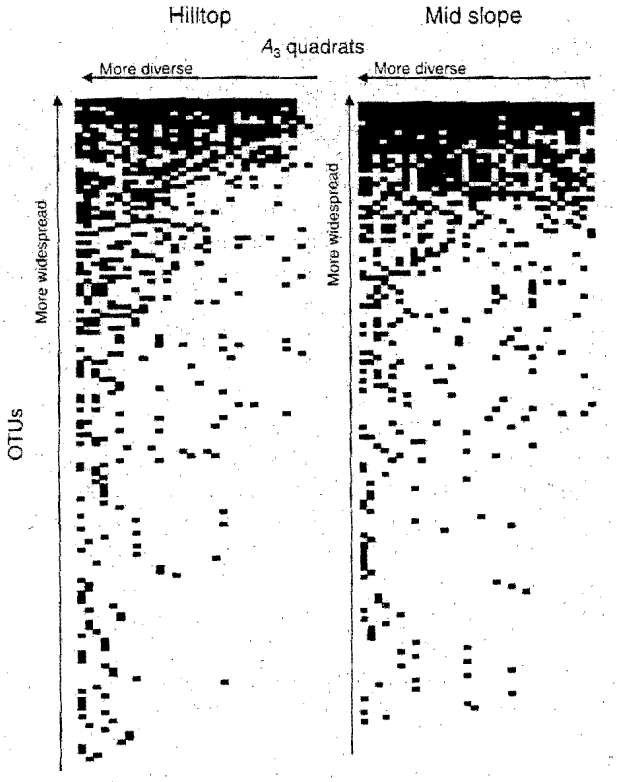


**Figure 4** Highly nested pattern of distribution of soil prokaryotic diversity in two 64-m² locations in the tropical dry forest of western Mexico, hilltop (left), slope (right). OTUs ranked according to their occupancy (widespread taxa close to the top, restricted taxa close to the bottom). Samples are ranked by their OTU diversity (richer sites close to the right extreme, less diverse sites close to the left extreme). Each point represents the presence of a given OTU in a given sample. In a perfectly nested pattern, points would arrange in a triangular pattern in such a way that the lower left part of the figure would contain no points.

### Nestedness

Both of our sampling sites showed a high degree of nestedness as measured using Atmar and Patterson's (1995) temperature calculator (Fig. 4). The hilltop location had a temperature value of $T = 12.55°$ [$P(T < 12.55) = 1.09 \times 10^{-78}$] and temperature at the mid-slope measured at $T = 25.05$ [$P(T < 25.05) = 7.56 \times 10^{-55}$]. $P$ values are the probabilities of temperatures equal or lower than the one observed, based on the distribution of $T$ values for randomly generated assemblages (Atmar & Patterson, 1995). In both locations, the nestedness values show that our prokaryotic assemblages are highly structured, clearly departing from values corresponding to random communities. In our locations, samples containing OTUs that occur in only one or very few samples are also the most diverse, thus generating the highly nested patterns. This result is consistent with the suggestion that microbial communities reflect adaptation to local environmental heterogeneity and are assemblages of generalist and specialist taxa (Balser *et al.*, 2002). Additionally, our results suggest that functions of microbial taxa are rarely interchangeable and are direct responses to environmental heterogeneity, as reported for macro-organisms. Moreover, these results demonstrate a non-random structure for prokaryotic assemblages, thus rejecting prediction 6 of the syndrome of species with no biogeography.

## CONCLUSIONS

Our analyses of the prokaryotic communities of two locations have allowed us to reject multiple criteria exhibited by organisms with no biogeography. Still, it could be argued that our finding of highly structured assemblages is merely a local pattern, not necessarily rejecting the ubiquity hypothesis in a biogeographical scale. That is, the possibility could remain that soil bacteria had a global dispersal but occurred locally only in suitable microenvironments, thus showing structured local communities but no biogeography. This possibility is unlikely in our sites, however, as 45.5% of taxa were exclusive to one site or the other, suggesting a non-random arrangement at the between-sites scale. Because we only sampled two sites, a direct test of prediction 1 of the syndrome of ubiquity is not reasonable. However, our results clearly contrast with those used to document the ubiquity of microbial eukaryote species that have relied on similarly small sample sizes (Finlay & Clark, 1999; Finlay, 2002; Fenchel, 2003).

We contend that it is inappropriate to think of bacteria as organisms that have an exceptional ecology or biogeography. Prokaryotic species assemblages, both in laboratory and natural conditions, have proved to be adequate model systems for testing ecological questions (Bohannan *et al.*, 2002; Jessup *et al.*, 2004; Srivastava *et al.*, 2004). We argue that the same can be stated for biogeographical matters. Our data show that rules that determine the distribution of vertebrates at a continental scale can be applied to prokaryotes in a 64-m² quadrat. Thus, we contend that a biogeography for prokaryotes is possible at such small scales, and that we can talk about OTU ranges of only a few metres in size. As it is the case with vertebrates at the continental scale, the ecological and evolutionary processes that determine the patterns documented here are not yet clearly established. What is clear is that soil prokaryotes do not belong to the set of organisms with no biogeography, as suggested by previous studies (Finlay, 2002).

## ACKNOWLEDGEMENTS

We thank Luis E. Eguiarte for ideas and review; Aldo Valera, Laura Espinosa, Maria Schneider, Stephen Bent and Mayee Wong, for technical assistance; and Salvador Araiza, Abel Verduzco, Manuel Montaño and Carlos Anaya for their help during the fieldwork. This project was supported by NASA NCC2-1051 and the Consejo Nacional de Ciencia y Tecnología, México CONACyT-G27674-N.

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## BIOSKETCHES

**Ana M. Noguez** is conducting a project on biogeochemical processes, soil bacterial diversity, and community structure of the tropical deciduous forest of the west coast of Mexico.

**Héctor Arita** is interested in the scaling of biological diversity and its links with the structure and dynamics of geographical ranges.

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**Larry Forney** is an expert on the ecology of prokaryotic organisms, with emphasis on the adaptive evolution in various species to assess the role of competition in determining the structure and diversity of microbial communities.

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# CAPÍTULO V

# CONCLUSIONES GENERALES

## CONCLUSIONES GENERALES

El análisis realizado en esta tesis incluyó diferentes aspectos, desde la exploración sobre la distribución espacial de las comunidades bacterianas, a una escala local; hasta el sondeo del papel de la estructura de dichas comunidades en los procesos funcionales del suelo, específicamente en la dinámica del C y N.

- Dinámica del C y N asociada a dos tamaños de agregados

Los resultados más importantes nos indican que: (a) los macro-agregados, en general presentan menores concentraciones de nutrientes que los micro-agregados; (b) el tipo de C encontrado en las dos fracciones es de naturaleza diferente, los macro-agregados poseen C menos procesado y por lo tanto más estable; (c) la tasa de pérdida de carbono lábil es menor en los macro-agregados que en micro-agregados; (d) el nitrógeno también presenta una redistribución y pérdida diferencial entre macro- y micro-agregados, siendo mucho más estable en los primeros; y (e) durante la época de lluvias la energía para las poblaciones microbianas es obtenida preferentemente a través de la transformación del amonio, principalmente en los macro-agregados.

- Agregados del suelo y comunidades bacterianas

Los resultados más importantes de este capítulo nos indican que las comunidades bacterianas presentes en los dos tamaños de partículas del suelo son de naturaleza diferente, así como los procesos de transformación de nutrientes que realizan dentro de cada fracción. Los resultados sugieren que los micro-agregados poseen una mayor proporción de organismos heterótrofos que estimulan la inmovilización del nitrógeno, protegiendo a este elemento de su pérdida por lixiviación en la época más húmeda; en contraste, en los macro-agregados dominan las bacterias nitrificantes.

Otro de los aspectos interesantes es el hecho de que aunque el 50% de los OTUs encontrados se localizan en ambos tamaños de agregados, existen OTUs exclusivos para macro- y micro-agregados (25%, respectivamente) y aunque estos son los menos abundantes son los que muy probablemente estén

marcando la diferencia en cuanto a la dinámica del C y N. Podemos aventurarnos a señalar que aunque en algunos casos las redundancias metabólicas existen, también existen organismos cuyos papeles en la dinámica de nutrientes están claramente definidos, sin importar cuán abundantes sean. El concepto de que las bacterias se encuentran en todas partes y de que sus funciones son intercambiables comienza a ser más obsoleto cada vez, como lo indican nuestros resultados.

- Distribución Espacial

En relación al tercer capítulo sobre la distribución espacial encontramos que a una escala local los organismos estudiados presentan:
(a) rangos de distribución reducidos con un alto porcentaje de "endémicos"; (b) valores de diversidad ß muy por encima de los que originalmente se suponía para estos organismos; (c) una curva "especie-área", en la cual el número de "especies" (OTUs) se incrementa con el área muestreada; y (d) comunidades no distribuidas al azar, es decir, estructuradas localmente. Es importante enfatizar que nuestros resultados demuestran que las mismas reglas que determinan la distribución de vertebrados a una escala continental se pueden aplicar a procariontes en un cuadrante de 64 $m^2$. Lo cual sugiere que existen patrones de distribución espacial comunes a la mayoría de los seres vivos y que su principal diferencia radica en la escala a la que se expresan de acuerdo con su forma de vida.

- Perspectivas

El estudio de los patrones de distribución y diversidad bacterianas es un área que se ha empezado a explorar muy recientemente; sin embargo, las posibilidades teóricas que esta aproximación nos plantea van desde la unificación de los estudios de ecología microbiana (incluyendo procesos de dispersión, especiación y extinción) hasta la búsqueda de patrones de diversidad y los procesos que determinan dichos patrones.

Nuestro trabajo indica que la aproximación macroecológica y biogeoquímica en el estudio de las comunidades microbianas en el suelo nos va abriendo puertas en la resolución de los papeles funcionales y de distribución

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de estos organismos. Aunque en este caso comenzamos a obtener ciertas respuestas, son aún más las preguntas que quedan en el aire. No hay que perder de vista que con nuestros resultados podemos plantearnos preguntas más concretas: ¿cuáles son los organismos específicos que están llevando a cabo la transformación del C y en especial del N?, ¿son los mismos en los macro-agregados que en los micro-agregados? o ¿cual es el papel de las meta-comunidades el la estructuración de comunidades a nivel local? o más aun ¿la relación especie área se mantiene a escalas mayores? Es indudable que el conciliar diferentes disciplinas realmente nos llevará a abrir la caja negra de la diversidad bacteriana y sus papeles funcionales.