LAND-USE SYSTEMS AFFECT ARCHAEAL COMMUNITY STRUCTURE AND FUNCTIONAL DIVERSITY IN WESTERN AMAZON SOILS[1]

Acácio Aparecido Navarrete[2], Rodrigo Gouvêa Taketani[3], Lucas William Mendes[4], Fabiana de Souza Cannavan[2], Fatima Maria de Souza Moreira[5] & Siu Mui Tsai[6]

SUMMARY

The study of the ecology of soil microbial communities at relevant spatial scales is primordial in the wide Amazon region due to the current land use changes. In this study, the diversity of the Archaea domain (community structure) and ammonia-oxidizing Archaea (richness and community composition) were investigated using molecular biology-based techniques in different land-use systems in western Amazonia, Brazil. Soil samples were collected in two periods with high precipitation (March 2008 and January 2009) from Inceptisols under primary tropical rainforest, secondary forest (5–20 year old), agricultural systems of indigenous people and cattle pasture. Denaturing gradient gel electrophoresis of polymerase chain reaction-amplified DNA (PCR-DGGE) using the 16S rRNA gene as a biomarker showed that archaeal community structures in crops and pasture soils are different from those in primary forest soil, which is more similar to the community structure in secondary forest soil. Sequence analysis of excised DGGE bands indicated the presence of crenarchaeal and euryarchaeal organisms. Based on clone library analysis of the gene coding the subunit of the enzyme ammonia monooxygenase (*amo*A) of Archaea (306 sequences), the Shannon-Wiener function and Simpson's index showed a greater ammonia-oxidizing archaeal

---

[1] Part of the Master's Thesis of the first author for the Applied Ecology Program of the University of Sao Paulo (USP). Work supported by grants from FAPESP, CNPq and GEF/UNEP. Received for publication in December 15, 2010 and approved in June 27, 2011.

[2] Master's Degree, Graduate Program in Applied Ecology of the Luiz de Queiroz College of Agriculture – ESALQ. University of Sao Paulo – USP. E- mails: navarrete@cena.usp.br; cannavan@cena.usp.br

[3] Pos-Doctor, Cell and Molecular Biology Laboratory, CENA-USP. E-mail: rgtaketani@yahoo.com.br

[4] Master's Degree, Graduate Program in Science of the Centre for Nuclear Energy in Agriculture – CENA. University of Sao Paulo – USP. E- mail: lwmendes@cena.usp.br

[5] Associate Professor of Soil Microbiology and Biochemistry, Soil Science Department, Federal University of Lavras – UFLA. CEP 37200-000, Lavras (MG). E-mail: fmoreira@dcs.ufla.br

[6] Full Professor at Cell and Molecular Biology Laboratory, CENA. University of Sao Paulo – USP. Av. Centenário 303, CEP 13400-970 Piracicaba (SP) Brazil. E-mail: tsai@cena.usp.br

diversity in primary forest soils (H' = 2.1486; D = 0.1366), followed by a lower diversity in soils under pasture (H' = 1.9629; D = 0.1715), crops (H' = 1.4613; D = 0.3309) and secondary forest (H' = 0.8633; D = 0.5405). All cloned inserts were similar to the Crenarchaeota *amo*A gene clones (identity ≥ 95 %) previously found in soils and sediments and distributed primarily in three major phylogenetic clusters. The findings indicate that agricultural systems of indigenous people and cattle pasture affect the archaeal community structure and diversity of ammonia-oxidizing *Archaea* in western Amazon soils.

Index terms: soil microbial ecology; soil microbiology; microbial diversity; land use changes; tropical soils.

RESUMO: *SISTEMAS DE USO DA TERRA AFETAM A ESTRUTURA DE COMUNIDADE E A DIVERSIDADE FUNCIONAL DE Archaea EM SOLOS DA AMAZÔNIA OCIDENTAL*

*O conhecimento acerca da ecologia de comunidades microbianas do solo em relevantes escalas espaciais é primordial na ampla região da Amazônia devido às mudanças de uso da terra. Neste estudo, a diversidade do Domínio Archaea (estrutura da comunidade) e Archaea oxidadora de amônia (riqueza e composição da comunidade) foi investigada usando-se técnicas moleculares em solos sob diferentes sistemas de uso da terra na Amazônia ocidental brasileira. Amostras de solo foram coletadas em dois períodos distintos de elevada intensidade pluviométrica (março de 2008 e janeiro de 2009) em Cambissolos sob floresta primária, floresta secundária (5 a 20 anos de regeneração), sistemas agrícolas de povos indígenas e pastagem. A eletroforese em gel com gradiente desnaturante de produtos amplificados pela reação em cadeia da polimerase (PCR-DGGE) usando o gene 16S rRNA como marcador filogenético indicou que as estruturas de comunidades de Archaea em solos sob cultivo agrícola e pastagem são diferentes daquelas encontradas em solos sob floresta primária. Estas, por sua vez, foram mais similares às estruturas de comunidades em solos sob floresta secundária. A análise de sequências de bandas excisadas de géis de DGGE revelou a presença de organismos dos filos Crenarchaeota e Euryarchaeota. Com base em análise de bibliotecas de clones do gene que codifica para a subunidade A da enzima amônia monoxigenase (amo*A *) de Archaea (306 sequências), os índices de Shannon-Wiener e Simpson mostraram maior diversidade de Archaea oxidadora de amônia em solos sob floresta primária (H' = 2,1486; D = 0,1366), seguido por menor diversidade em solos sob pastagem (H' = 1,9629; D = 0,1715), cultivo agrícola (H' = 1,4613; D = 0,3309) e floresta secundária (H' = 0,8633; D = 0,5405). Todos os insertos clonados foram similares a clones do gene amo*A *de Crenarchaeota (identidade ≥95 %) previamente detectados em solos e sedimentos; e distribuíram-se em três agrupamentos filogenéticos principais. Os resultados indicaram que a estrutura de comunidades de Archaea e a diversidade de Archaea oxidadora de amônia na Amazônia ocidental são afetadas em solos sob sistemas agrícolas de povos indígenas e pastagem.*

Termos para indexação: ecologia microbiana do solo; microbiologia do solo; diversidade microbiana; mudança de uso da terra; solos tropicais.

INTRODUCTION

Archaea constitute one of the three major evolutionary lineages of life on Earth (Woese, 1987; Woese et al., 1990). For the last two decades, Archaea have been the focus of a growing number of studies on microbial ecology due to their ubiquity and abundance in various natural and anthropogenically influenced terrestrial ecosystems, such as arable land, grassland and forest soils (reviewed by Chaban et al., 2006). Some Archaea carry the ammonia monooxygenase subunit A (*amo*A) gene (Venter et al., 2004), which can be up to 3.000-fold more abundant than bacterial *amo*A genes in soil (Leininger et al., 2006). The ubiquity of archaeal ammonia oxidizers has been indicated in multiple habitats, such as oxic and suboxic marine water bodies, estuarine sediments, temperate soils (Francis et al., 2005) and Amazonian anthrosols (Taketani & Tsai, 2010).

The Amazon contains the world's largest area of contiguous tropical forest and is constantly affected by high rates of deforestation and conversion to cattle pasture and other agricultural uses. In this context, Brazil has been slowing down deforestation by trying

to make better use of already cleared land (Tollefson, 2010). However, the establishment of policies for the sustainable use of land in the Amazon region requires considering not only the diversity of flora and fauna but also the aspects of microbial ecology and biogeochemistry represented in this region as well (Piccolo et al., 1994; Borneman & Triplett, 1997; Moraes et al., 2002; Cenciani et al., 2009; Chaves et al., 2009; Jesus et al., 2009; O'Neill et al., 2009; Pazinato et al., 2010).

Although members of soil Archaea are difficult to culture, molecular studies have demonstrated the presence of archaeal 16S rRNA gene sequences in agricultural, grassland and forest soils, but these were dominated by sequences typical of Crenarchaeota (Bintrim et al., 1997; Jurgens et al., 1997). Recent molecular studies have increased indications for the importance of archaeal ammonia oxidizers in the nitrogen cycle (reviewed by Jetten, 2008; Prosser & Nicol, 2008; Schauss et al., 2009), thus compelling us to investigate the effect of different land-use systems on archaeal communities and, more specifically, ammonia oxidizers in tropical soils.

The aim of this study was to assess the effects of current land-use systems on the genetic and functional diversity of Archaea in soils of different landscape types in the Brazilian western Amazon that were previously covered with native tropical rainforest. Polymerase chain reaction (PCR) amplification targeting the universal archaeal 16S rRNA gene and a further denaturing gradient gel electrophoresis (DGGE) fingerprinting method were used to determine the community structure of soil Archaea. Archaeal *amo*A gene clone libraries were used to evaluate the richness and community composition of ammonia-oxidizing Archaea.

MATERIALS AND METHODS

Field sites

The studied sites are located in Benjamin Constant, a municipality in the State of Amazonas, Brazil, (between 4°35' and 4°42' S and 69°60' and 70°01' W), along the Solimões River (Figure 1). The climate in the region is classified as Af (Köppen's classification), with an annual average temperature of 25.7 °C and an average precipitation of 2562 mm (Coelho et al., 2005). At all sampling points considered in this study, the soil type was Inceptisol (Coelho et al., 2005). The deforestation rate in this region is low, mainly due to the difficulty of access and low population density.

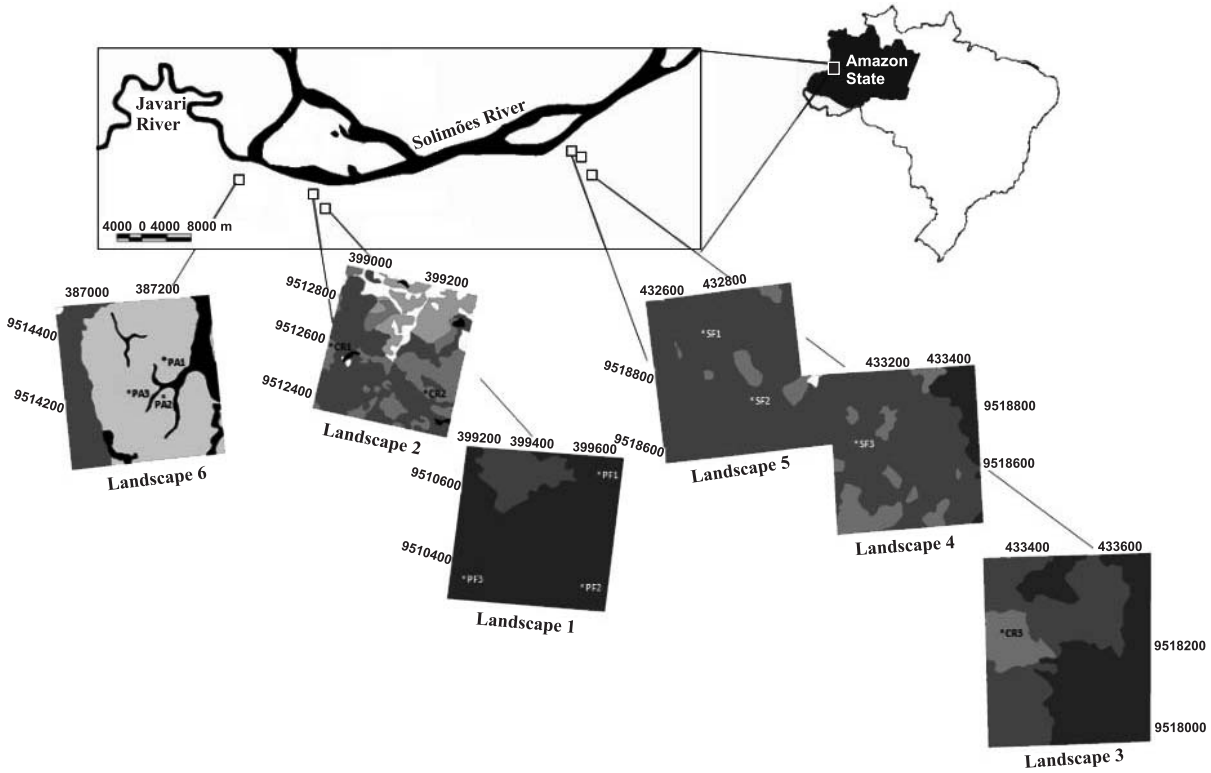

Figure 1. Geographical location of the studied landscapes in the municipality of Benjamin Constant, Amazonia, Brazil, on the Solimões River. Sampling points (•) were located in different land use systems which are distinguished by gray-levels: primary forest (PF) [■], secondary forest (SF) [■], crops (CR) [■] and pasture (PA) [■]. Exposed soil [□] and water [■] were present in the landscapes 6 and 2, respectively. (Land-use maps adapted from Fidalgo et al., 2005).

The Amazonian landscapes were identified by satellite images and selected according to the vegetation cover, soil use and management practices (Table 1, Figure 1). The dominant use and land cover in the six different landscapes studied were: primary forest, secondary forest, crops and pasture. In this region, the agricultural systems of indigenous people are primarily based on slash-and-burn, annual crops in shifting cultivation and a long fallow period, which involves the abandonment of areas to allow natural regeneration which involves a fallow period for approximately three years (Fidalgo et al., 2005). Some pasture areas are also present as a consequence of governmental policies implemented in the 1970s. Amendments, fertilizers and pesticides were not applied in any of the land-use systems.

Sampling procedure and DNA extraction

Soil was sampled in the rainy seasons, in March 2008 and January 2009. Samples were collected from the 0–20 cm topsoil layer using a cylindrical sampler (diameter 5 cm) after removing the litter layer. Three soil samples were collected from each land use system considered in this study – primary forest, secondary forest (5 to 20 year old), local traditional crops and pasture. All sites were originally highland native forest. Immediately after sampling, the soil was ice-cooled for transport and frozen at -20 °C. The samples were processed within 72 h after sampling. Soil DNA was extracted from 250 mg sample (total humid weight) using the Power Soil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA), according to the manufacturer's instructions. The DNA extraction reactions were performed in triplicate for each soil sample.

PCR amplification of the archaeal 16S rRNA gene and DGGE analysis

For analysis by DGGE, the extracted DNA was amplified by a nested PCR approach using the archaeal 16S rRNA gene primers 21F and 958R (DeLong, 1992) in the first round, and the primers 340F-GC and 519R (Øvreås et al., 1997) in the second. Each 25 µL reaction mixture contained approximately 10 ng of template DNA, 1 × PCR buffer (Invitrogen,

Table 1. Previous and actual soil use at each sampling point in Benjamin Constant, upper Solimões River region, western Amazon, Brazil

Land use	Landscape	Sampling point	Latitude/longitude	Previous soil use[1]	Soil use 2008/2009
Primary forest	1	FP1 FP2 FP3	4°42'70"S/69 °90'54"W 4°42'95"S/69 °90'58"W 4°42'93"S/69 °90'84"W	Primary forest	Primary forest
Crops	2	CR1	4°40'85"S/69 °91'05"W	Secondary forest until 2007, followed by slash -and -burn	Cassava and banana cultivation. Plant residues incorporated
	2	CR2	4°40'99"S/69 °98'81"W	Cassava cultivation until 2007, abandoned from 2007 to 2008 when the area was cleared again	Cassava cultivation
	3	CR3	4°35' 80"S/69°60'08"W	Secondary forest until 2007, followed by slash -and -burn and cassava cultivation	Cassava cultivation. Plant residues incorporated (trunks and branches of trees)
Secondary forest	4	SF1	4°35'27"S/69 °60'61"W	Annual crops in shifting cultivation. Secondary forest since 2007	Old secondary forest. *Gramineae* on the soil surface
	5	SF2	4°35'35"S/69 °60'52"W	Annual crops in shifting cultivation. Secondary forest since 2007	Old secondary forest. Plant family Heliconiacea in the understory
	5	SF3	4°35'42"S/69 °60'31"W	Annual crops in shifting cultivation. Secondary forest in regeneration since 1993	Secondary forest in advanced stage of regeneration
Pasture	6	PA1 PA2 PA3	4°39'35"S/70 °01'63"W 4°39'45"S/70 °01'63"W 4°39'46"S/70 °01'65"W	Forest until 1945, followed by sugarcane cultivation until the 70s when the area was used as pasture (*Brachiaria brizantha*, *Brachiaria humidicola* and *Paspalum notatum flugge*)	Pasture. Beef cattle

[1] Fidalgo et al. (2005).

Carlsbad, CA), 3.0 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} of each deoxynucleoside triphosphate, 0.5 µmol L^{-1} of each forward (F) and reverse (R) primer, and 1.0 U of Taq DNA polymerase (Invitrogen). The PCR mixtures were incubated in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, CA, USA) for the following amplification cycles: 5 min at 95 °C; 30 cycles of 30 s at 95 °C; 30 s at 53 °C (first round); and 55.8 °C (second round); 1 min at 72 °C; followed by 10 min at 72 °C.

The PCR products, approximately 179 bp long, were analyzed by denaturing gradient gel electrophoresis (PCR-DGGE) on 6 % (w/v) polyacrylamide gel with a linear chemical gradient ranging from 35 to 55 % (100 % denaturant, 7 M urea, and 40 % v/v formamide). Aliquots of 1200 ng concentrated PCR products were loaded into wells of the DGGE gel and electrophoresed in 0.5 × TAE buffer at 60 °C with 175 V for 5 h, using the DGGE apparatus PhorU2 (Ingeny, Leiden, the Netherlands). After electrophoresis, the gels were washed for 30 min (in a solution of 10 % v/v ethanol and 0.5 % v/v acetic acid), stained for 20 min in a solution (0.2 % w/v $AgNO_3$), washed in developing solution for 10 min (1.5 % w/v sodium hydroxide and 0.8 % v/v formaldehyde), washed in fixing solution for 5 min, and photographed.

The detected bands were analyzed with the Bionumerics program (Applied Mathematics, Kortrijk, Belgium), using the UPGMA algorithm (unweighted pair-group method, with an arithmetic mean) (Sneath & Sokal, 1973) and the Jaccard coefficient. The tolerance position was set at 1 % and background subtraction was applied. Both strong and weak bands were included in the analysis, thus taking into account the presence and absence of bands at specific positions. The band richness was expressed as the total number of detectable 16S rRNA gene amplicons on the DGGE profiles. The analysis of similarities (ANOSIM) for 16S rRNA gene DGGE patterns was performed considering both band presence and absence.

Selected DGGE bands were excised, macerated in sterile water and subjected to a further PCR amplification using primers 340F and 519R (Øvreås et al., 1997). The new PCR products were purified with GFX PCR DNA and the Gel Band Purification Kit (Amersham Pharmacia Biotech, NJ, USA) and cloned into a pGEM-T Easy Vector (Promega, WI, USA) according to the manufacturer's instructions. Plasmids were isolated from *Escherichia coli* DH5α using standard protocols. The purified plasmids with the correct insert (length was evaluated on agarose gels) were then sequenced in both directions with universal M13 primers. Sequence analysis (GenBank accession nos. GQ847667-GQ847674) was performed using the alignment and classification tools of the Greengenes database (http://greengenes.lbl.gov). A neighbor-joining phylogenetic tree was constructed based on the alignment of nucleotide sequences using MEGA 4.0 (Tamura et al., 2007). Bootstrap analysis was performed with the above settings with 100 replicates. For the phylogenetic tree, 10 sequences selected from the GenBank database (sequences from agricultural, mesophilic and alkaline-saline soil and marine sediment) were included.

Amplification and sequencing of archaeal *amo*A genes

The DNA extracted from the soil sampled in March 2008 was pooled for each land use system and archaeal *amo*A gene fragments (635 bp) were amplified using the PCR primers Arch-amoAF and Arch-amoAR (Francis et al., 2005). Each 25 µL reaction mixture contained approximately 50 ng of template DNA, 1 × PCR buffer (Invitrogen, Carlsbad, CA), 5.0 mmol L^{-1} $MgCl_2$, 0.8 mmol L^{-1} concentration of each deoxynucleoside triphosphate, 0.3 µmol L^{-1} of each forward (F) and reverse (R) primer, and 1.25 U of Taq DNA polymerase (Invitrogen). The PCR mixtures were incubated in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) for the following amplification cycles: 95 °C for 5 min; 30 cycles consisting of 94 °C for 45 s; 53 °C for 60 s; and 72 °C for 60 s; and 72 °C for 10 min. The PCR products were purified using the Qiagen PCR purification kit (Qiagen, Valencia, CA, USA) after analysis by gel electrophoresis. The PCR products were ligated into the pGEM®-T Easy vector (Promega, Madison, WI, USA) and transformed into competent *E. coli* DH5α cells. A total of 306 clones were randomly selected and sequenced. Sequencing was performed using the vector primers M13F and M13R (Huey & Hall, 1989), the DYEnamic ET Terminator Cycle Sequencing Kit (GE Healthcare, NJ, USA) and an ABI PRISM 3100 Genetic Analyzer capillary sequencer (Applied Biosystems). Libraries were constructed for the samples from primary forest (GenBank accession nos. GQ480941-GQ481023), crops (GenBank accession nos. GQ481024-GQ481095), pasture (GenBank accession nos. GQ481096-GQ481172) and secondary forest (GenBank accession nos. GQ481173-GQ481246) sites. All sequences were compared with those in the GenBank database using the BLASTn program.

Measuring diversity, and phylogenetic and statistical analyses

The nucleotide sequences were assembled and edited using Phred/Phrap/Consed (Ewing et al., 1998; Gordon et al., 1998) and nucleotide alignments were generated using ClustalX2 (Larkin et al., 2007). Operational taxonomic units (OTUs) were defined as sequence groups in which sequences differed by ≤ 5 % (Beman & Francis, 2006). Diversity measures – including rarefaction analysis, ACE, Chao1 and Jackknife nonparametric richness estimates, the Shannon-Wiener function and Simpson's index – were performed using MOTHUR (Schloss et al., 2009), based on the nucleotide sequences.

The original nucleotide sequences were translated into amino acid sequences using the CLC Sequence

Viewer 6.0.2 software (CLC bio). A neighbor-joining phylogenetic tree (based on the Dayhoff PAM residue subtraction matrix and 1000 bootstraps) was constructed based on the alignment of one representative amino acid sequence of each OTU using ARB Software Environment for Sequence Data (Ludwig et al., 2004) and a graphical presentation was created using iTOL (Letunic & Bork, 2007). For the phylogenetic tree, 29 sequences obtained from the GenBank database were included, which were translated into amino acid sequences (14 representative agricultural soil sequences, 12 sequences from marine sediments, 1 sequence from an activated sludge bioreactor and 2 sequences from a geothermal mine). The reference sequences were chosen to show the diversity of the sequences and to indicate the closest relatives to the sequences found in our study.

The community of archaeal ammonia oxidizers was also examined using UniFrac (Lozupone & Knight, 2005); this program compares phylogenetic information. Both the UniFrac distance metric and the *P* test were used to make comparisons of the compositional overlap between archaeal *amoA* clone libraries. A neighbor-joining phylogenetic tree including all 306 sequences recovered in this study was used as input file for UniFrac. The UniFrac *P* values were based on comparisons with 1000 randomized trees. Principal coordinate analyses (PCoA) were performed using the UniFrac branch length metric to separate the clone libraries.

RESULTS

Soil chemical properties

The soil chemical properties did not show significant differences ($p > 0.05$) among the samples collected under primary forest, secondary forest, crops or pasture regarding the properties analyzed in the two-year sampling period (Table 2). However, subtle differences between land-use systems were detected. The soil pH was lower in the forests than under crops and pasture. The organic matter (OM) content in soils under crops and secondary forest was higher than at the sites under primary forest and pasture. The soil $N\text{-}NH_4^+$ concentration was lower in soil under secondary forest than under other land-use systems. Subtle differences were also noted in the soil $N\text{-}NO_3^-$ and total N concentrations under different land-use systems, although the $N\text{-}NO_3^-$ concentrations were lower in primary forest soils.

DGGE analysis of archaeal community structure and sequence analysis of excised DGGE bands

Complex archaeal community fingerprints were observed with the 16S rRNA marker gene, consisting of dominant bands against a background of numerous fainter bands. The DGGE profile comparisons were designed to identify the effect of each land use system in relation to the natural forest soils (i.e. primary forest soil). Hence, soil from each of the different land uses was run on a single gel with primary forest samples and these gels were then used for cluster analysis (Figure 2). The ANOSIM's *R* values indicated that the communities in crops and pasture soils were clearly separated ($R > 0.75$) from those in primary forest soil. However, the archaeal communities observed in primary and secondary forest soils were barely separable (Figure 2).

In the cluster analysis of the PCR-DGGE groups, the banding patterns associated with triplicate PCR products analyzed by DGGE for the same sampling point clustered with 70–100 % similarity. The banding patterns obtained at three different sampling points associated with the same land use system

Table 2. Soil chemical characteristics of the 0–20 cm topsoil layer under different land-use systems in Benjamin Constant, a municipality on the upper Solimões River, western Amazon region

Land use		pH $CaCl_2$	Organic matter[1]	$N\text{-}NH_4^+$[2]	$N\text{-}NO_3^-$[3]	Total N[4]
			g dm^{-3}	mg kg^{-1} N		g kg^{-1}
Primary forest	March 2008	4.0 ± 0.4	29.0 ± 14.7	63.1 ± 2.0	2.0 ± 0.7	2.0 ± 0.2
	January 2009	4.1 ± 0.2	32.3 ± 5.2	61.9 ± 3.0	2.1 ± 0.5	2.0 ± 0.3
Secondary forest	March 2008	3.9 ± 0.05	34.7 ± 20.3	45.5 ± 3.0	2.7 ± 1.3	2.0 ± 0.3
	January 2009	4.0 ± 0.3	26.3 ± 3.9	40.5 ± 2.0	2.5 ± 1.5	2.0 ± 0.4
Crops	March 2008	4.8 ± 0.8	39.3 ± 15.5	66.0 ± 4.0	2.4 ± 0.4	2.0 ± 0.7
	January 2009	4.4 ± 0.6	25.7 ± 2.9	64.0 ± 3.0	2.5 ± 0.8	2.0 ± 0.6
Pasture	March 2008	4.5 ± 0.6	27.3 ± 9.2	71.5 ± 2.1	2.9 ± 1.1	1.9 ± 0.3
	January 2009	3.8 ± 0.1	31.3 ± 5.5	72.7 ± 3.4	3.1 ± 1.2	1.9 ± 0.5

[1] Walkley-Black method. [2] Electrical conductivity method. [3] Kjeldahl digestion method. [4] Kjeldahl method. The values are averages based on triplicate sampling points of each land-use system. Standard deviations are shown in the table.

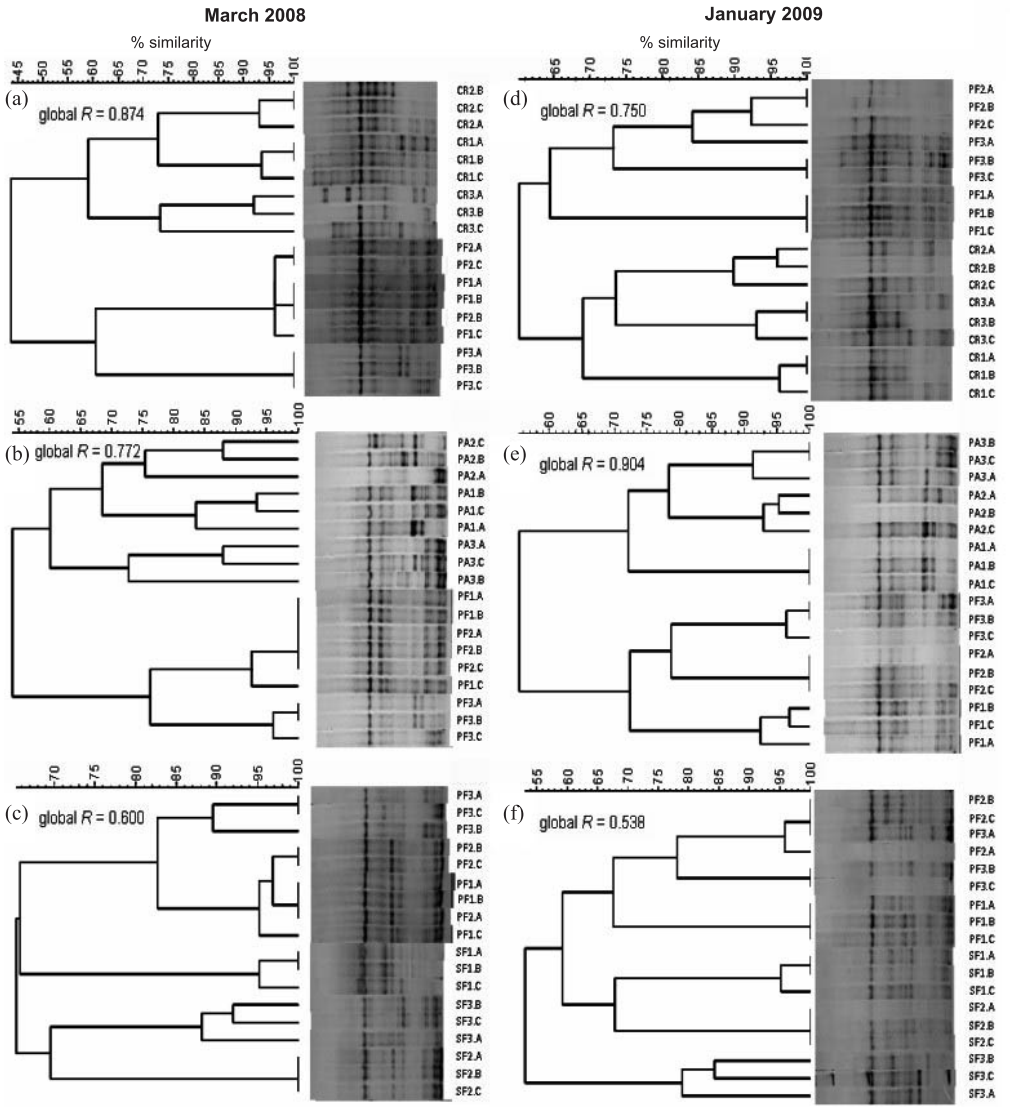

Figure 2. Inferred similarity of dominant archaeal community structures using 16S rRNA DGGE profiles after cluster analysis with the UPGMA algorithm and the Jaccard coefficient. Each spot in the fingerprint represents 16S rRNA sequences with different melting points. ANOSIM test results (*R* values) are shown for each comparison. (PF) Primary forest; (CR) Crops; (PA) Pasture; (SF) Secondary forest. A, B and C refer to triplicate PCR products analyzed by DGGE.

clustered at > 60 % similarity. The PCR-DGGE products clustered at 45 % (March 2008, Figure 2a) and 60 % (January 2009, Figure 2d) similarity when archaeal community structures from primary forest soil were compared to crop soil DGGE profiles. The DGGE profiles from primary forest soil clustered at 55 % (March 2008; Figure 2b) and 55 % (January 2009; Figure 2e) similarity when compared to the pasture soil DGGE profiles, and at 65 % (March 2008; Figure 2c) and 59 % (January 2009; Figure 2f) when compared to the secondary forest soil DGGE profiles.

Following the PCR-DGGE analysis (Figure 3), no differences were observed in band richness between land-use systems regarding the 2008 samples. For the 2009 samples, band richness was greater in the primary forest soil and at the sites under crops than under pasture and secondary forest soils.

The dendrograms showed common and specific bands in each sample group. The identification of 10 DGGE bands (Figure 3) confirmed the specificity of the bands amplified with Archaea-specific primers once members of the Crenarchaeota and Euryarchaeota phyla were identified. The BLASTn similarity searches were performed on all 10 sequences to identify the closest related sequences reported in other studies. Most sequences showed high sequence similarity to other cloned archaeal 16S rRNA gene sequences (identity ≥ 96 %). Archaea bands 2, 4 and 10 were also found to be only distantly related to other Archaea in the database (88, 86 and 86 % identities,

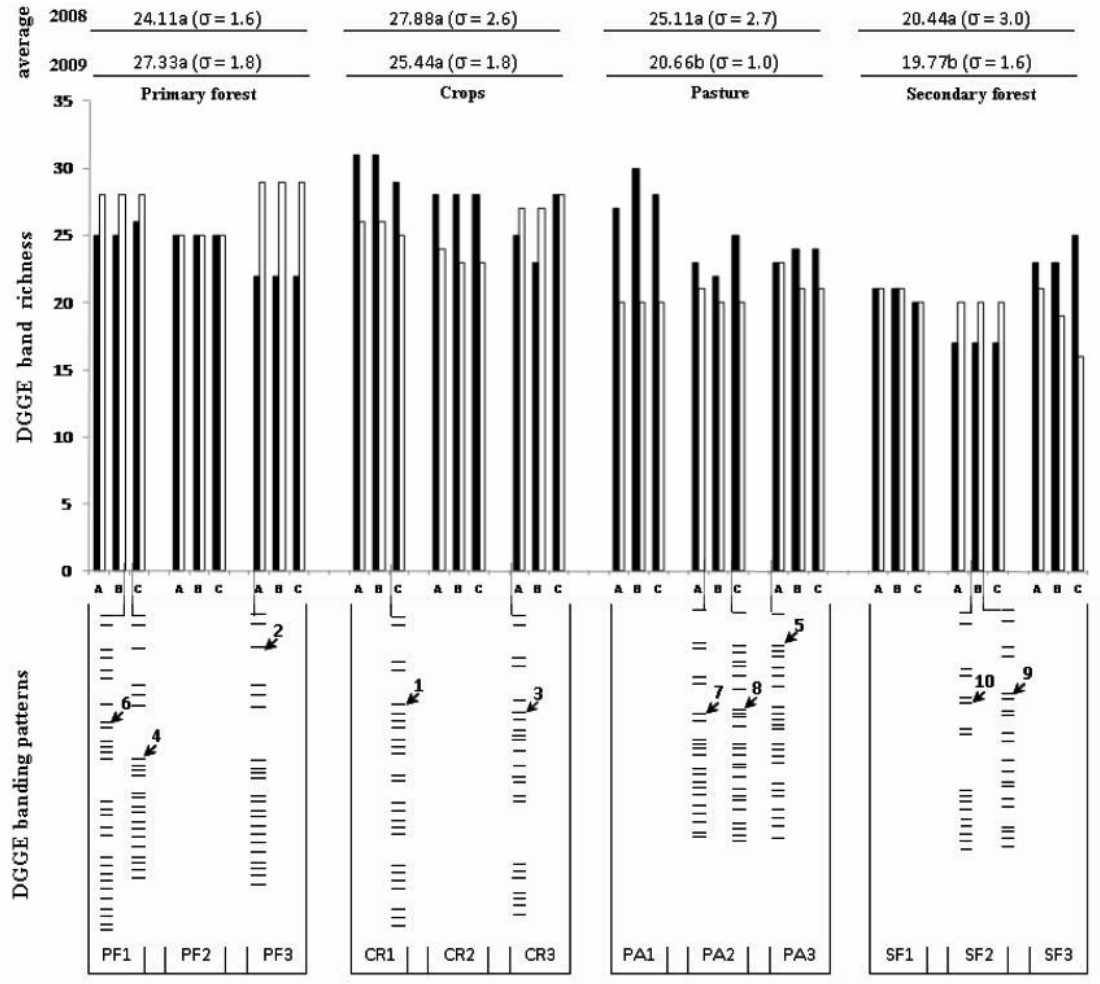

Figure 3. Richness of archaeal communities as indicated by the DGGE banding patterns from triplicate samples of soil from four different land-use systems (primary forest, crops, pasture and old secondary forest) in Benjamin Constant. Columns in black and white indicate the two samplings - March 2008 and January 2009, respectively. A, B and C refer to the PCR products analyzed in triplicate by DGGE. The DGGE banding patterns are only shown for the samples with sequenced bands. The bands indicated by an arrow were excised from the gel, re-amplified and sequenced. σ = standard deviation. Land-use systems indicated by the same letter (a, b) were not significantly different ($p < 0.05$) by Tukey´s test and performed independently for the two sampling periods.

respectively). Phylogenetic analysis placed all 10 sequences within the cloned archaeal 16S rRNA gene sequences, with each sequence falling into three phylogenetic clusters primarily comprising sequences retrieved from agriculture, mesophilic and alkaline-saline soils and marine sediments (Figure 4). Cluster A included crenarchaeotal clones (identity ≥ 88 %) originating from all four land-use systems (bands 2, 3, 6, 7, 8 and 9). The nearest neighbors to cluster A sequences were clones obtained from agricultural and mesophilic (turf field) soils. Cluster B included two crenarchaeotal clones (*Candidatus nitrososphaera*; 86 % identity) originating from crops and pasture soils (bands 1 and 5). Cluster C was most distinct from clusters A and B and included two euryarchaeotal clones (86 % identity) originating from primary and secondary forest soils (bands 4 and 10). The sequences nearest cluster C were clones obtained from marine sediments.

Ammonia monooxygenase subunit A (*amo*A) gene sequence analysis

Of the gene encoding a subunit of the key enzyme ammonia monooxygenase (*amo*A) of Archaea present in the primary forest, secondary forest, crops and pasture soils, 306 sequences were recovered. BLASTn data analysis showed that all cloned inserts were similar to Crenarchaeota ammonia monooxygenase subunit A (*amo*A) gene clones (identity ≥ 95 %). Most of these cloned sequences were most similar to sequences recovered from tropical marine sediments by Dang et al. (2009) and agricultural soils by He et al. (2007).

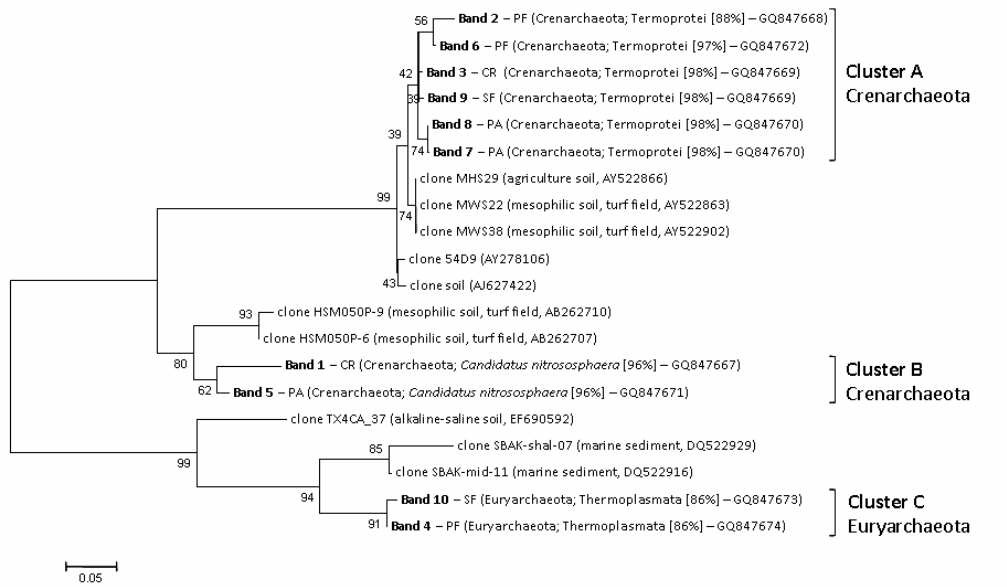

Figure 4. Relationships of the unrooted neighbor-joining phylogenetic tree between archaeal sequences obtained from sequencing purified fragments from DGGE gels. The 16S rRNA gene sequences were classified by alignment against the Greengenes database. Numbers next to branches refer to the results of the bootstrap analysis. The numerical values in brackets indicate the percentage identity. (PF) primary forest; (CR) crops; (PA) pasture; (SF) secondary forest.

From these 306 individual archaeal *amo*A sequences, a total of 36 unique OTUs (based on a 5 % cutoff point) was recovered. OTU richness was only lower in primary forest soils, when compared to the library from pasture soils (Table 3, Figure 5). Rarefaction curves for the libraries of soils under primary and secondary forest reached an asymptote at 11 OTUs and 4 OTUs, respectively. The curve plotted for the library from crop soil tended to reach an asymptote after 9 OTUs; however, the curve for the library from pasture soils suggested the existence of unsampled OTUs.

The richness estimates are presented in table 3 with the heterogeneity measures calculated by the Shannon-Wiener function and Simpson's index. The ACE, Jackknife and Chao1 nonparametric richness estimates showed the differences in the OTU richness of the archaeal *amo*A gene libraries numerically, by the rarefaction curves. The high values expressed by the Jackknife estimator for the libraries from the primary forest and pasture soils (17.42 and 17.00) were due to the numbers of unique OTUs (represented by only one archaeal *amo*A gene sequence) found in these libraries.

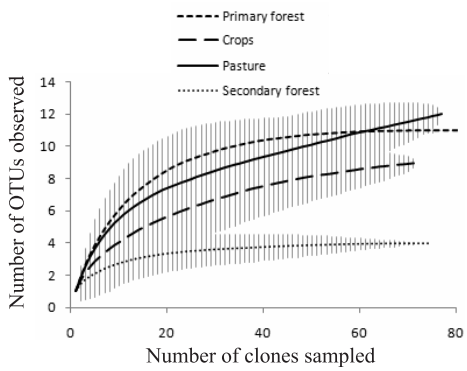

Figure 5. Rarefaction curves indicating archaeal *amo*A richness within clone libraries derived from primary forest, secondary forest, crop and pasture soils. The OTUs were defined as groups of sequences that differed by ≤ 5 % at the DNA level.

Table 3. Archaeal *amo*A richness estimates and heterogeneity measures based on a 5 % cutoff point in the total archaeal *amo*A sequences from primary forest, secondary forest, crop and pasture soils

Land use	OTUs richness			Heterogeneity measures	
	ACE	Chao1	Jackknife	Shannon - Wiener	Simpson
Primary forest	11.00	11.00	17.42	2.1486	0.1366
Crops	10.28	9.33	10.43	1.4613	0.3309
Pasture	23.80	17.00	17.00	1.9629	0.1715
Secondary forest	4.00	4.00	4.00	0.8633	0.5405

The values calculated for the Shannon-Wiener function and Simpson's index indicated a greater diversity of ammonia-oxidizing Archaea in primary forest soils (H' = 2.1486; D = 0.1366, respectively), followed by a lower diversity of this functional microbial group in the soils under pasture (H' = 1.9629; D = 0.1715), crops (H' = 1.4613; D = 0.3309) and secondary forest (H' = 0.8633; D = 0.5405), consecutively.

Not only was diversity altered, but the composition of the archaeal ammonia-oxidizing communities was changed as well, as shown by pairwise comparisons (p = 0.00). The result of the principal coordinate analysis is shown in figure 6. Graphically, the ordination of the clone libraries showed that the soil ammonia-oxidizing archaeal communities differed between land-use systems. The data variation in percent explained by the ordination axes was 79.87 % (PC1=50.58 %).

In the phylogenetic analysis of the PCR clone libraries, the archaeal *amo*A gene sequences recovered in this study formed two phylogenetic clades with GenBank sequences recovered from sediments and soils; they did not cluster according to land-use systems (Figure 7). Most of the sequences clustered in three major protein clusters (clusters A, B and C); the exceptions were two clones (GenBank accession nos. GQ481040 and GQ481041) both recovered from crop soils. Cluster A was most distinct from clusters B and C and may have been specific to crops and pasture soils. Cluster B included sequences originating from marine sediments, agricultural soil and clones retrieved in this study that originated from primary forest and pasture soils. Interestingly, cluster C included clones originating from all four land-use systems.

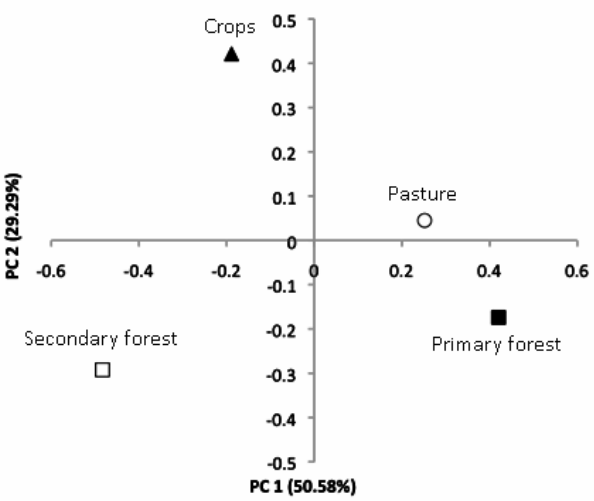

Figure 6. Principal component analysis (PCoA) resulting in UniFrac statistical comparison of the ammonia-oxidizing archaeal communities based on phylogenetic compositions: pairwise UniFrac distances calculated from a neighbor-joining phylogenetic tree.

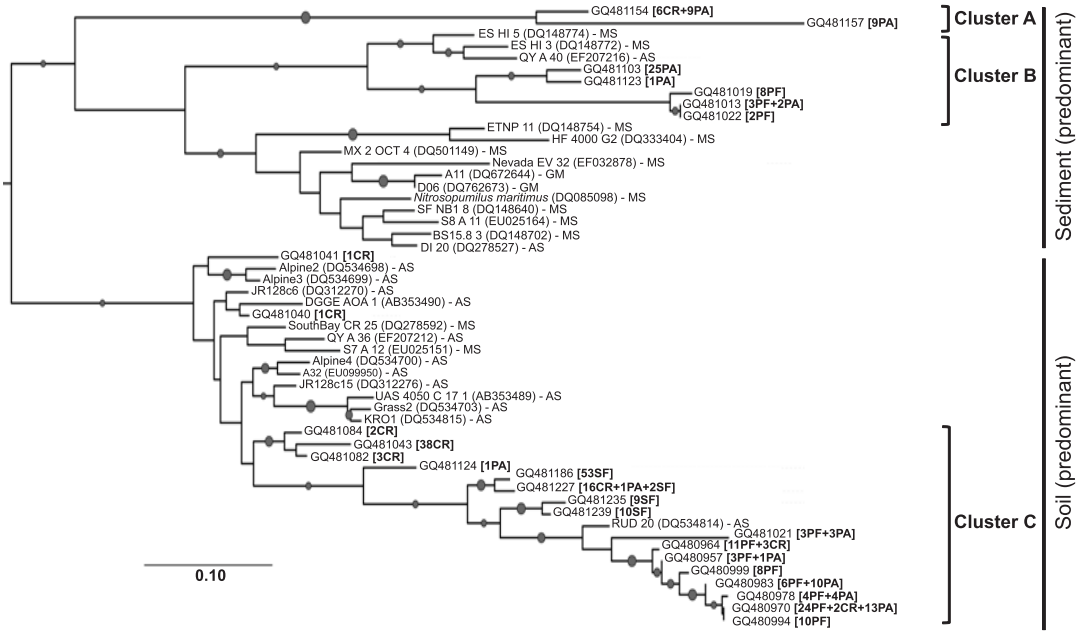

Figure 7. Unrooted neighbor-joining tree showing phylogenetic relationships between archaeal *amo*A sequences originating from soils under different land-use systems (PF: primary forest, CR: crops, PA: pasture and SF: secondary forest [this study]), agricultural soil (AS), marine sediment (MS), geothermal mine (GM) and the pure-culture ammonia-oxidizing archaeon *Nitrosopumilus maritimus*. Bootstrap confidence levels are shown in circles. Bootstrap values are indicated by filled-in circles on the branch points. Numerical values and abbreviations in brackets indicate the number of clones in each OTU (5 % cutoff point of dissimilarity) and their origins, respectively.

DISCUSSION

The present study assessed the soil archaeal communities, including ammonia-oxidizing Archaea, in current land-use systems in discontinuous areas (landscapes) of an approximate area of 54 ha in the Brazilian western Amazon (Coelho et al., 2005). In previous studies, these land-use systems were significantly differentiated by chemical properties of the topsoil (0–20 cm) based on an extensive list of properties and greater number of sampling points of the Inceptisols (Coelho et al., 2005; Moreira et al., 2009). The chemical properties of the soil samples evaluated in this study (Table 2) indicated the same tendency, however they did not show significant differences between land-use systems using Tukey's test. These areas were originally highland forest (Fidalgo et al., 2005), however due to differences regarding land use and intensity of management they can be considered a mosaic landscape. The natural soil cover in the Amazonian landscapes is tropical rainforest; therefore, one can assume that the composition of the soil archaeal communities before cultivation or pasture would be similar to that found in the current primary forest.

The crop sites studied here have relatively recent cropping history: one year since the beginning of the current cultivation (Table 1). Nevertheless, the differences in ammonia-oxidizing archaeal communities observed between crop and primary forest soils were highly significant; the diversity in primary forest soil tended to be higher. Several authors have previously studied the effect on soil after forest removal to make room for crops. Moreira et al. (2009) studied the same soils considered in this study regarding their fertility and showed that agricultural soils are mainly related to high base saturation, exchangeable bases and Ca and Mg. Juo & Manu (1996) reported that in slash-and-burn agriculture, soil properties change as a consequence of the deposition of ash from the burned vegetation and increased rates of organic matter decomposition. Over time, nutrients become depleted and these properties tend to return to their previous state. Interestingly, Yrjala et al. (2004) showed that the addition of ashes to the soil microcosm leads to significant changes in the archaeal community. In this context, it is also important to consider that different plant development stages, litter quality and historical soil management practices can influence the diversity and community structure of soil-inhabiting Archaea (Hai et al., 2009; Su et al., 2010).

Based on the *amo*A gene sequences analysis, our results indicated a high richness of ammonia-oxidizing Archaea in pasture soil. However, a higher diversity was detected in soil under long-standing primary forest. The community composition of ammonia-oxidizing Archaea also differed between pasture and primary forest soils. Although $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ were slightly higher in pasture soil, our pastures had not received additional inputs through the application of N fertilizer, nor did they show significant differences when compared to primary forest regarding soil chemical and physical properties (Table 2). Cattle pastures normally receive high N inputs through urine deposition of by grazing animals. Several studies have indicated that animal urine patches are the major source of N leaching into grazed pastures (Ledgard et al., 1999; Zaman & Blennerhassett, 2010). The urea in urine is rapidly converted into NH_4^+ and then to NO_3^-, a process known as nitrification, by the activities of soil microorganisms. These conditions are likely to have supported a greater richness in ammonia-oxidizing Archaea communities in pasture soil. Similar results for ammonia-oxidizing Archaea communities were shown by O'Callaghan et al. (2010) in response to the addition of urine to soil.

Harris (2003) and Nogueira et al. (2006) reported that measurements of the soil microbial community can be used as indicators of sustainability in many agroecosystems and in the evaluation of the impact of reforestation. The molecular data from the DGGE analysis of 16S rRNA for the Archaea domain showed that soil archaeal communities tended to become more similar to those of the primary forest when the forest was allowed to regenerate in an area under controlled agricultural use. Recent results from the same Amazon soils as in this study showed that archaeal and bacterial communities, evaluated by automated ribosomal intergenic spacer analysis (ARISA) and terminal restriction fragment length polymorphism (T-RFLP), respectively, presented similar ecological patterns to the archaeal communities in this investigation (Jesus et al., 2009; Navarrete et al., 2010). Interestingly, Moreira et al. (2009) reported that secondary forest is similar to primary forest regarding the fertility of the Inceptisols in the upper Solimões river region.

The phylogenetic analysis of PCR clone libraries of archaeal *amo*A gene sequences indicated a high similarity between archaeal *amo*A gene sequences from secondary forest soils and sequences recovered from crop, pasture and primary forest soils (Cluster C, Figure 7). The selection and adaptation of particular phylogenetic lineages (Nugroho et al., 2007) can potentially reflect the effects of long-term differences in growth and colonization of ammonia-oxidizing Archaea populations in the studied soils under forest regeneration. The natural fluctuations in the soil archaeal communities between the two sampling periods could not be explained, due to the inherent difficulties of a simultaneous analysis of the variability of multiple populations with relative abundance as a function of natural influences (e.g., temperature, plant growth, rainfall) over a time period.

CONCLUSIONS

1. Based on DGGE fingerprinting of the archaeal 16S rRNA gene amplified from soil DNA and archaeal

gene clone library analysis of ammonia monooxygenase subunit A (*amo*A), archaeal community structure and diversity of ammonia-oxidizing Archaea in soils under agricultural systems of indigenous people and cattle pasture are not similar to those in soils under the current primary tropical rainforest.

2. Soil archaeal communities tend to become similar to those of primary forest when the forest is allowed to regenerate.

3. DGGE fingerprinting and clone libraries (16S rRNA and *amo*A genes) are useful indexes for insights into the ecology of soil archaeal communities (community structure, richness and community composition) under different land use systems and at relevant spatial scales in the western Amazon region.

4. A focus for future investigations would be long-term studies of re-colonization processes of archaeal populations in soils under regenerating forest in Amazon soils.

ACKNOWLEDGEMENTS

This work was supported by a M.Sc. scholarship from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo - 07/01868-3). The authors are indebted to Mr. José Elias Gomes and Dra. Mariana Gomes Germano for their technical and scientific support. We acknowledge the Global Environmental Facility/United Nations Environment Program (GEF/UNEP project CSM-BGBD/GF2715-02, BiosBrasil project), FAPESP Thematic Project (08/58114-3) and Conselho Nacional de Desenvolvimento Científico (CNPq) for grants and research fellowships to S. M. Tsai and F. M. S. Moreira.

LITERATURE CITED

BEMAN, J.M. & FRANCIS, C.A. Diversity of ammonia-oxidizing archaea and bacteria in the sediments of a hypernutrified subtropical estuary: Bahia del Tobari, Mexico. Appl. Environ. Microbiol., 72:7767-7777, 2006.

BINTRIM, S.B.; DONOHUE, T.J.; HANDELSMAN, J.; ROBERTS, G.P. & GOODMAN, R.M. Molecular phylogeny of archaea from soil. Proc. Nat. Acad. Sci. USA., 94:277-282, 1997.

BORNEMAN, J. & TRIPLETT, E.W. Molecular microbial diversity in soils from Eastern Amazonia: Evidence for unusual microorganisms and microbial population shifts associated with deforestation. Appl. Environ. Microbiol., 63:2647-2653, 1997.

BREMNER, J.M. Total nitrogen. In Methods of Soil Analysis, ed. C.A. Black, D.D. Evans, L.J. White, L.E. Ensminger, and Clark F.E. (Am. Soc. Agron. Madison WI, 1965), p.1149.

CENCIANI, K.; LAMBAIS, M.R.; CERRI, C.C.; DE AZEVEDO, L.C.B. & FEIGL, B.J. Bacteria diversity and microbial biomass in forest, pasture and fallow soils in the Southwestern Amazon Basin. R. Bras. Ci. Solo, 33:907-916, 2009.

O'CALLAGHAN, M.; GERARD, E.M.; CARTER, P.E.; LARDNER, R.; SARATHCHANDRA, U.; BURCH, G.; GHANI, A. & BELL, N. Effect of the nitrification inhibitor dicyandiamide (DCD) on microbial communities in a pasture soil amended with bovine urine. Soil Biol. Biochem. 42:1425-1436, 2010.

CHABAN, B.; NG, S.Y.M. & JARRELL, K.F. Archaeal habitats – From the extreme to the ordinary. Can. J. Microbiol., 52:73-116, 2006.

CHAVES, J.; NEILL, C.; GERMER, S.; GOUVEA-NETO, S.; KRUSCHE, A.V.; BONILLA, A.C. & ELSENBEER, H. Nitrogen transformations in flowpaths leading from soils to streams in Amazon forest and pasture. Ecosystems, 12:961-972, 2009.

COELHO, M.R.; FIDALGO, E.C.C.; ARAÚJO, F.O.; DOS SANTOS, H.G.; MENDONÇA SANTOS, M.L.; PÉREZ, D.V. & MOREIRA, F.M.S. Solos das áreas-piloto do Projeto BiosBrasil (Conservation and Sustainable Management of Below Ground Biodiversity: Phase I), Município de Benjamim Constant, Estado do Amazonas. B. Pesq. Desenvol., 67:1-159, 2005. Disponível em: www.biosbrasil.ufla.br or http://www.cnps.embrapa.br/solosbr/publicacoes/levantamentodesolos. Accesso em: 16 set. 2010.

DANG, H.; LI, J.; ZHANG, X.; LI, T.; TIAN, F. & JIN, W. Diversity and spatial distribution of *amo*A-encoding archaea in the deep-sea sediments of the tropical West Pacific Continental Margin. J. Appl. Microbiol., 106:1482-1493, 2009.

DeLONG, E.F. *Archaea* in coastal marine environments. Proc. Nat. Acad. Sci. USA., 89:5685-5689, 1992.

EIGENBERG, R.A.; DORAN, J.W.; NIENABER, J.A.; FERGUSON, R.B.; WOODBURY, B.L. Electrical conductivity monitoring of soil condition and available N with animal manure and a cover crop. Agric. Ecosyst. Environ., 88:183-193, 2002.

EWING, B.; HIJJER, L.; WENDL, M.C. & GREEN, P. Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. Genome Res., 8:175-185, 1998.

FIDALGO, E.C.C.; COELHO, M.R.; ARAÚJO, F.O.; MOREIRA, F.M.S.; SANTOS, H.G.; DOS SANTOS, M.L.M. & HUISING, J. Levantamento do uso e cobertura da terra de seis áreas amostrais relacionadas ao projeto BiosBrasil (Conservation and Sustainable Management of Below-Ground Biodiversity: Phase I), Município de Benjamin Constant (AM). B. Pesq. Desenvol., 67:1-15, 2005.

FRANCIS, C.A.; ROBERTS, K.J.; BEMAN, J.M.; SANTORO, A.E. & OAKLEY, B.B. Ubiquity and diversity of ammonia oxidizing archaea in water columns and sediments of the ocean. Proc. Nat. Acad. Sci. USA, 102:14683-14688, 2005.

GORDON, D.; ABAJIAN, C. & GREEN, P. Consed: A geographical tool for sequence finishing. Genome Res., 8:195-202, 1998.

HAI, B.; DIALLO, N.H.; SALL, S.; HAESLER, F.; SCHAUSS, K.; BONZI, M.; ASSIGBETSE, K.; CHOTTE, J-L.; MUNCH, J.C. & SCHLOTER, M. Quantification of key genes steering the microbial nitrogen cycle in the rhizosphere of sorghum cultivars in tropical agroecosystems. Appl. Environ. Microbiol., 75:4993-5000, 2009.

HARRIS, J.A. Measurements of the soil microbial community for estimating the success of restoration. Europ. J. Soil Sci., 54:801-808, 2003.

HE, J.Z.; SHEN, J.P.; ZHANG, L.M.; ZHU, Y.G.; ZHENG, Y.M.; XU, M.G. & DI, H. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. Environ. Microbiol., 9:2364-2374, 2007.

HUEY, B. & HALL, J. Hypervariable DNA fingerprint in *Escherichia coli*: Minisatellite probe from bacteriophage M13. J. Bacteriol., 171:2528-2532, 1989.

JESUS, E.C.; MARSH, T.L.; TIEDJE, J.M. & MOREIRA, F.M.S. Changes in land use alter the structure of bacterial communities in Western Amazon soils. ISME J., 3:1004-1011, 2009.

JETTEN, M.S.M. The microbial nitrogen cycle. Environ. Microbiol., 10:2903-2909, 2008.

JUO, A.S. & MANU, R.A. Chemical dynamics in slash-and-burn agriculture. Agric. Ecosyst. Environ., 58:49-60, 1996.

JURGENS, G.; LINSTROM, K. & SAANO, A. Novel group within the kingdom *Crenarchaeota* from boreal forest soil. Appl. Environ. Microbiol., 63:803-805, 1997.

LARKIN, M.A.; BLACKSHIELDS, G.; BROWN, N.P.; CHENNA, R.; MCGETTINGAN, P.A.; MCWILLIAM, H.; VALENTIN, F.; WALLACE, I.M.; WILM, A.; LOPEZ, R.; THOMPSON, J.D.; GIBSON, T.J. & HIGGINS, D.G. ClustalW2 and ClustalX version 2. Bioinformatics, 23:2947–2948, 2007.

LEDGARD, S.F.; PENNO, J.W. & SPROSEN, M.S. Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer application. J. Agric. Sci., 132:215-225, 1999.

LEININGER, S.; URICH, T.; SCHLOTER M.; SCHWARK, L.; QI, J.; NICOL, G.W.; PROSSER, J.I.; SCHUSTER, S.C. & SCHLEPER, C. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature, 442:806-809, 2006.

LETUNIC, I. & BORK, P. Interactive tree of life (iTOL): An online tool for phylogenetic tree display and annotation. Bioinformatics, 23:127-128, 2007.

LOZUPONE, C.A. & KNIGHT, R. UniFrac: A new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol., 71:8228-8235, 2005.

LUDWIG, W.; STRUNK, O.; WESTRAM, R.; RICHTER, L.; MEIER, H.; YADHUKUMAR, B.A.; LAI, T.; STEPPI, S.; JOBB, G.; FÖRSTER, W.; BRETTSKE, I.; GERBER, S.; GINHART, A.W.; GROSS, O.; GRUMANN, S.; HERMANN, S.; JOST, R.; KÖNIG, A.; LISS, T.; LÜSSMANN, R.; MAY, M.; NONHOFF, B.; REICHEL, B.; STREHLOW, R.; STAMATAKIS, A.; STUCKMANN, N.; VILBIG, A.; LENKE, M.; LUDWIG, T.; BODE, A. & SCHLEIFER, K.H. ARB: A software environment for sequence data. Nucl. Acids. Res., 32:1363-1371, 2004.

MORAES, J.F.L.; NEILL, C.; VOLKOFF, B.; CERRI, C.C.; MELILLO, J.; LIMA, V.C. & STEUDLER, P.A. Soil carbon and nitrogen stocks following forest conversion to pasture in the Western Brazilian Amazon Basin. Acta Sci., 24:1369-1376, 2002.

MOREIRA, F.M.S.; NÓBREGA, R.S.A.; JESUS, E.C.; FERREIRA, D.F. & PÉREZ, D.V. Differentiation in the fertility of Inceptisols as related to land use in the upper Solimões river region, western Amazon. Sci. Total Environ., 408:349-355, 2009.

NAVARRETE, A.A.; CANNAVAN, F.S.; TAKETANI, R.G. & TSAI, S.M. A molecular survey of the diversity of microbial communities in different Amazonian agricultural model systems. Diversity, 2:787-809, 2010.

O'NEILL, B.; GROSSMAN, J.; TSAI, M.T.; GOMES, J.E.; LEHMANN, J.; PETERSON, J.; NEVES, E. & THIES, J.E. Bacterial community composition in Brazilian anthrosols and adjacent soils characterized using culturing and molecular identification. Microbiol. Ecol., 58:23-35, 2009.

NOGUEIRA, M.A.; ALBINO, U.B.; BRANDÃO-JUNIOR, O.; BRAUN, G.; CRUZ, M.F.; DIAS, B.A.; DUARTE, R.T.D.; GIOPPO, N.M.R.; MENNA, P.; ORLANDI, J.M.; RAIMAM, M.P.; RAMPAZO, L.G.L.; SANTOS, M.A.; SILVA, M.E.Z.; VIEIRA, F.P.; TOREZAN, J.M.D.; HUNGRIA, M. & ANDRADE, G. Promising indicators for assessment of agroecosystem alterations among natural, reforested and agricultural land use in southern Brazil. Agric. Ecosyst. Environ., 115:237-247, 2006.

NUGROHO, R.A.; RÖLING, W.F.M.; LAVERMAN, A.M. & VERHOEF, H.A. Low nitrification rates in acid Scots pine forest soils are due to pH-related factors. Microbiol. Ecol., 53:89-97, 2007.

ØVREÅS, L.; FORNEY, L.; DAAE, F.L. & TORSVIK, V. Distribution of bacterioplankton in meromictic Lake Saelevannet, as determined by denaturing gradient gel eletrophoresis of PCR amplified gene fragments coding for 16S rDNA. Appl. Environ. Microbiol., 63:3367-3373, 1997.

PAZINATO, J.M.; PAULO, E.N.; MENDES, L.W.; VAZOLLER, R.F. & TSAI, S.M. Molecular characterization of the archaeal community in an Amazonian wetland soil and culture-dependent isolation of methanogenic *Archaea*. Diversity, 2:1026-1047, 2010.

PICCOLO, M.C.; NEILL, C. & CERRI, C.C. Natural abundance of 15N in soils along forest-to-pasture chronosequences in the Western Brazilian Amazon Basin. Oecologia, 99:112-117, 1994.

PROSSER, J.I. & NICOL, G.W. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. Environ. Microbiol., 10:2931-2941, 2008.

SCHAUSS, K.; FOCKS, A.; LEININGER, S.; KOTZERKE, A.; HEUER, H.; THIELE-BRUHN, S.; SHARMA, S.; WILKE, B.M.; MATTHIES, M.; SMALLA, K.; MUNCH, J.C.; AMELUNG, W.; KAUPENJOHANN, M.; SCHLOTER, M. & SCHLEPER, C. Dynamics and functional relevance of ammonia-oxidizing archaea in two agricultural soils. Environ. Microbiol., 11:446–456, 2009.

SCHLOSS, P.D.; WESTCOTT, S.L.; RYABIN, T.; HALL, J.R.; HARTMANN, M.; HOLLISTER, E.B.; LESNIEWSKI, R.A.; OAKLEY, B.B.; PARKS, D.H.; ROBINSON, C.J.; SAHL, J.W.; STRES, B.; THALLINGER, G.G.; van HORN, D.J. & WEBER, C.F. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol., 75:7537-7541, 2009.

SNEATH, P.H.A. & SOKAL, R.R. Numerical taxonomy: The principles and practice of numerical classification. San Francisco, W.H. Freeman & Co, 1973.

SU, M.; KLEINEIDAM, K. & SCHLOTER, M. Influence of different litter quality on the abundance of genes involved in nitrification and denitrification after freezing and thawing of an arable soil. Biol. Fert. Soils, 46:537-541, 2010.

TAKETANI, R.G. & TSAI, S.M. The influence of different land uses on the structure of archaeal communities in Amazonian anthrosols based on 16S rRNA and amoA genes. Microbiol. Ecol. 59:734-743, 2010.

TAMURA, K.; DUDLEY, J.; NEI, M. & KUMAR, S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molec. Biol. Evol., 24:1596-1599, 2007.

TOLLEFSON, J. The global farm. Nature News, 466:554-556, 2010.

VENTER, J.C.; REMINGTON, K.; HEIDELBERG, J.F.; HALPERN, A.L.; RUSCH, D.; EISEN, J.A.; WU, D.; PAULSEN, I.; NELSON, K.E.; NELSON, W.; FOUTS, D.E.; LEVY, S.; KNAP, A.H.; LOMAS, M.W.; NEALSON, K.; WHITE, O.; PETERSON, J.; HOFFMAN, J.; PARSONS, R.; BADEN-TILLSON, H.; PFANNKOCH, C.; ROGERS, Y-H. & SMITH, H.O. Environmental genome shotgun sequencing of the Sargasso Sea. Science, 304:66-74, 2004.

WOESE, C.R. Bacterial evolution. Microbiol. Rev., 51:221-271, 1987.

WOESE, C.R.; KANDLER, O. & WHEELIS, M.L. Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eukarya. Proc. Nat. Acad. Sci. USA, 87:4576-4579, 1990.

YRJALA, K.; KATAINEN, R.; JURGENS, G.; SAARELA, U.; SAANO, A.; ROMANTSCHUK, M. & FRITZE, H. Wood ash fertilization alters the forest humus Archaea community. Soil Biol. Biochem., 36:199-201, 2004.

ZAMAN, M. & BLENNERHASSETT, J.D. Effects of different rates of urease and nitrification inhibitors on gaseous emissions of ammonia and nitrous oxide, nitrate leaching and pasture production from urine patches in an intensive grazed pasture system. Agric. Ecosyst. Environ. 136:236-246, 2010.