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BIOGEOGRAFIA E PERFIL DE DIVERSIDADE DE ARCHAEAS METANOGÊNICAS EM LAGOS TROPICAIS

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Vivam, vivam, vivam
Os montes, e a planície, e as ervas!
Vivam os rios, vivam as fontes!
Vivam as flores, e as árvores, e as pedras!
Vivam os entes vivos _ os bichos pequenos,
Os bichos que correm, insectos e aves,
Os animais todos, tão reais sem mim,
Os homens, as mulheres, as crianças,
As famílias, e as não-famílias, igualmente!
Tudo quanto sente sem saber porquê!
Tudo quanto vive sem pensar que vive!
Tudo que acaba e cessa sem angústia nem nada,
Sabendo melhor que eu, que nada há que temer,
Que nada é fim, que nada é abismo, que nada é mistério,
E que tudo é Deus, e que tudo é Ser, e que tudo é Vida.".

ÁLVARO DE CAMPOS (heterônimo de Fernando Pessoa)

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RESUMO

BARRETO, Davi Pedroni. Biogeography and diversity of methanogenic Archaea in tropical lakes. Rio de Janeiro, 2013. Dissertação (Mestrado em Ciências Biológicas - Microbiologia), Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2013.

O estudo da distribuição dos microrganismos através do espaço e do tempo nos permite observar seus padrões biogeográficos através de valores, como por exemplo, o índice espécie-area (z). Dado a grande habilidade dispersiva e reprodutiva, e ainda a baixa taxa de extinção dos microrganismos, eles tendem a ser amplamente distribuídos, e acredita-se que eles são selecionados principalmente por fatores ambientais contemporâneos. Estudos recentes demonstraram que, apesar destas características, em determinadas situações, microrganismos podem apresentar características típicas de macrorganismos no que diz respeito a sua distribuição. O objetivo deste estudo foi avaliar os padrões de distribuição de bactérias e archaeas em ambientes contínuos e relativamente homogêneos. Foram coletadas 26 amostras de sedimento de dois lagos localizados na Amazônia e no Pantanal, distribuídas em uma grade de distâncias de alcance de 0,01m até 1400m. As amostras foram analisadas por T-RFLP (*Terminal restriction fragment length polymorphism*) para os genes *mcrA* (Metil-coenzima M redutase, um gene importante no processo de metanogênese), 16S rRNA para Archaea e Bacteria. Os índices de similaridade e dissimilaridade foram correlacionados com as distâncias entres os pontos. Em ambos os ambientes estudados foi possível confirmar a existência de padrões biogeográficos, a partir de uma significativa relação espécie-área, descrita a partir de seu valor-z. O gene *mcrA* obteve os maiores valores de z tanto no lago do Pantanal quanto no Amazônico (0,014 e 0,009 respectivamente), Bacteria e Archaea também apresentaram resultados significativos no lago do Pantanal (0,009 e 0,015). Isto nos sugere que fatores relacionados à distância geográfica estão direcionando em algum nível a composição das comunidades microbianas nestes lagos. No lago amazônico, também foram avaliadas as taxas de metanogênese acetotrófica e hidrogenotrófica no sedimento, nos mesmos pontos em que os padrões biogeográficos foram avaliados. Os resultados demonstraram que, embora as taxas de metanogênese não diferirem significativamente entre os pontos de coleta, o coeficiente de variação das taxas diminui drasticamente dos pontos mais distantes para os mais próximos, sendo desta forma condizente com nossos resultados relativos à diversidade microbiológica. Neste estudo, nós demonstramos que microrganismos de comunidades metanogênicas, que são ativos em um ambiente relativamente contínuo e homogêneo, apresentam uma distribuição espacial não randômica, e uma relação taxa-área significativa. Isto também pode estar influenciando as taxas de transformação da matéria orgânica por meio de metanogênese nestes ambientes.

Palavras-chave: metanogênese; Pantanal; Amazônia; T-RFLP; relação espécie-área; microrganismos.

ABSTRACT

BARRETO, Davi Pedroni. Biogeography and diversity of methanogenic Archaea in tropical lakes. Rio de Janeiro, 2013. Dissertação (Mestrado em Ciências Biológicas - Microbiologia), Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2013.

The study of the distribution of microorganisms through space (and time) allows the evaluation of biogeographic patterns, such as the species-area index (z). Owing to their high dispersal ability, high reproduction rates, and low rates of extinction, microorganisms tend to be widely distributed, and are thought to be virtually cosmopolitan and selected primarily by environmental factors. Recent studies have shown that, despite these characteristics, microorganisms may behave like larger organisms and exhibit geographical distribution. In this study, we investigated patterns of spatial diversity distribution of bacteria and archaea in a relatively homogeneous environment. We collected 26 sediment samples from two different tropical lakes, distributed in a nested grid, with distances between samples ranging from 0.01 m to 1400 m. The samples were analyzed using terminal restriction fragment length polymorphism (T-RFLP) targeting the *mcrA* gene (coding for a subunit of methyl-coenzyme M reductase) and the genes of archaeal and bacterial 16S rRNA. From the qualitative and quantitative results (relative abundance of operational taxonomic units), we calculated the similarity index for each pair to evaluate the taxa-area and distance-decay relationship slopes by linear regression. In both the studied environments, it was possible to confirm, by analyses of taxa-area relationships and the z-value, the existence of a biogeography pattern. The *mcrA* gene presented higher z-values in both the Pantanal and Amazonia lakes (0.014 and 0.009, respectively). Bacteria and Archaea also showed significant results in the Pantanal lake (0.009 and 0.015). This suggests that geographic distance-related factors direct, at some level, the composition of the microbial communities in these lakes. Sediment incubations were also carried out with samples from the Grande do Curuai Lake, to measure the hydrogenotrophic and acetotrophic methanogenesis rates of the same points that were analyzed for diversity. Despite similar methane production rates in different quadrants, we were able to observe that distant sampling points presented larger rate variations, and consequently, a larger coefficient of variation. In this study, we showed that the microorganisms of a methanogenic community that is active in a continuous and relatively homogeneous environment display spatial distribution and a taxa-area relationship, and that this might influence the rate of methanogenic transformation of organic matter in these environments.

Keywords: methanogenesis; Pantanal; Amazon; T-RFLP; species-area relationship; microorganisms

1. INTRODUCTION

The concept of biogeography is defined as the study of the distribution and range of living organisms across space and time. It consists of scientific investigations that attempt to document and understand the distribution patterns of organisms, both past and present (BROWN & LOMOLILO, 1998; COX & MOORE, 2005). Most studies in this field have traditionally been performed targeting macroorganisms such as plants and animals (BEGON *et al.*, 2006). With the development of molecular tools, the concepts of biogeography are now also being studied in microorganisms. It has been long debated as to whether microorganisms have a ubiquitous distribution or patterns of biogeography, but recently a consensus for the existence of microbial biogeography is emerging (FENCHEL *et al.,* 1997; FENCHEL, 2003; MARTINY *et al.,* 2006).

Geographical isolation is a primary mechanism for speciation among macroorganisms; therefore, if there has been little or no biogeography in the evolutionary history of microorganisms, how did this group become so diverse? Clearly, the vast ancestral age of microorganisms on the planet, rapid generation times, and horizontal gene transfer all contribute to this diversity. Thus, it seems unlikely that microorganisms can be considered exempt from biogeographic distribution (ROUT & CALLAWAY, 2012)

1.1. Biogeography of microorganisms

A long-held concept in microbial ecology is that microorganisms are ubiquitously distributed and can be found in any habitat with favorable environmental conditions. This concept was introduced by Martinus Willem Beijerinck and concisely summarized by Lourens Gerhard Marinus Baas Becking in the quote “Everything is everywhere, the environment selects” (BAAS-BECKING, 1934). This statement is based on some traits of microorganisms, such as the small size of individuals and the consequent ease of their dispersal across long distances, high rates of reproduction,

low generation times, and large population sizes, leading to a small chance of local extinction.

Free-living eukaryotic microorganisms are often described as occurring ubiquitously. When they are not dominant in some specific environment, it is possible to reanimate the cryptic diversity by changing the *in vitro* environmental conditions (FENCHEL *et al.*, 1997; FINLAY, 2002). A study showed that it is possible to find nearly 80% of all known species of the flagellate genus *Paraphysomonas* in just a small sample of sediment (FINLAY & CLARKE, 1999), meaning that the high local diversity of this genus does not result in an even higher global diversity. The observation is mostly explained by the high dispersal rate (due the low size of the flagellates), extremely low generation times (leading to a low rate of extinction) and also the capacity to generate resistant forms when environmental conditions are unfavorable (FINLAY & CLARKE, 1999). The authors suggested that if eukaryotic microorganisms were ubiquitous, prokaryotic microorganisms should be ubiquitous as well, since they have an even smaller size and larger populations. Indeed, some studies on prokaryotes suggested global distribution—for example, psychrophilic polar bacteria were found at both the South and the North poles—however, data were not sufficient to gather strong evidence of a cosmopolitan distribution (STALEY & GOSINK, 1999).

Nevertheless, more recent studies have shown that the distribution of microorganisms is not random, and that biogeographic patterns of distribution are well established (PAPKE *et al.*, 2003; PAPKE & WARD, 2004). The distribution of total soil microbial biomass was showed to be not random, but rather predominantly related to water and nutrient availability in the environment, and this characteristic of the soil is able to explain, by modeling, 50% of the variability of the global microbial biomass (SERNA-CHAVEZ *et al.*, 2013).

In high altitude soils, changes in the diversity of specific clades in the composition of high alpine bacterial communities have been shown to be driven by spatial autocorrelation at low-scale distances. The same is not observed at larger geographical distances, indicating biogeographical distribution on a local scale. Soil pH, plant abundance, and snow depth were the most important variables structuring the diversity of these communities (KING *et al.*, 2010)

Even *Saccharomyces cerevisiae,* a well-known yeast that is extremely important for alcoholic fermentation and in the production of wine, has been shown to be not randomly or even ubiquitously distributed. Studies have shown that specific strains are closely related to the type of culture (organic or conventional), grape variety, and soil management. The combination of these different factors selects different phylogenetic populations within the species (TOFALO *et al.*, 2013).

Viruses also show biogeographical distribution patterns. Marine cyanophages that infect *Synechococcus sp.* from sites from the east coast of North America to the islands of the Bermudas were analyzed by molecular techniques and they presented substantially different community compositions (those from the Bermudas did not share any common taxa with those from North America) that correlated mostly with the sampled water body. Moreover, these cyanophages showed temporal variation in community composition that was driven strongly by seasonal factors (MARSTON *et al.*, 2013).

In the Arctic sea floor, bacterial communities showed patterns of biogeography related to geographical distances and the dynamics of oceanic water masses, which could act as geographical barriers to the dispersion of microorganisms and control the diversity of bacteria in the ocean. However, in the case of the Atlantic Ocean surface sediment, geographical isolation seems to play a less important role in the structure of the bacterial communities, with geographical distance and contemporary environmental factors being the most important drivers of a small, but significant, pattern of biogeography in this case (GALAND *et al.*, 2010; SCHAUER *et al.*, 2010).

Magnetotactic bacteria have also been shown to demonstrate biogeographical distribution, influenced by both environmental and geographical factors. Sediment samples from China and USA were compared with respect to their population diversity, and different factors were tested to explain the differential distribution. Temperature and salinity (and environmental factors in general) were shown to be the most significant causes of this biogeographical variation, and geographical distance and the geomagnetic field strength were also significant modulators (LIN *et al.*, 2013).

As another example, genetic distances between populations of microorganisms were shown to increase with geographic distance, which might represent allopatric speciation (DINIZ-FILHO & TELLES, 2000). On this subject, purple nonsulfur bacteria (*Rhodopseudomonas palustris*) from freshwater marsh sediments showed large genetic variability that was directly associated with geographical distance between sampling sites, coupled with significant changes in phenotypical characteristics (ODA *et al.*, 2003).

Further studies on the subject of allopatric speciation have employed a pangenomic approach to determine whether this process actually occurs and at what rate. By doing so, they have been able to determine that the geographical isolation of 7 different populations of the thermoacidophilic crenarchaeon *Sulfolobus islandicus* in 3 different regions was a determinant factor in the divergence of their core and variable genomes, by preventing gene flow among them (RENO *et al.*, 2009).

As an alternative to genotyping analyses, Lucio *et al.*, (2008) used metabolic compounds as a parameter of comparison between different populations of the extremophilic bacterium *Salinibacter rubium*. Furthermore, by using high-resolution mass spectrometry techniques, it was possible to compare the phenotypes of the microorganisms to reveal biogeographical discrimination. They discovered that different phenotypes were related to different geographical locations, and that the divergence among them was related to the geographical distance between the sites.

Other studies have been able to identify endemic microorganisms and true geographic isolation in extreme environments such as hot springs, pristine soils, salt lakes, and hot and cold deserts around the world, all of which form strong evidence for non-cosmopolitan distribution. Fluorescent *Pseudomonas* isolated from undisturbed pristine soil in 38 sampling sites from 4 different continents has been shown to present high endemicity levels when analyzed by the BOX-A1R-based repetitive extragenic palindromic polymerase chain reaction (BOX-PCR) DNA fingerprinting technique, and the dissimilarities among sites and continents showed positive correlation with the geographical distances between them (CHO & TIEDJE, 2000).

Hot spring cyanobacteria sampled from 4 different locations (Japan, New Zealand, Italy, and North America) presented a high correlation between community composition dissimilarity and geographical distance; however, it seems that environmental and spring geochemical characteristics also structure microbial diversity in these habitats. It was also possible to access sequences that were unique to the North American springs, suggesting that the geographical distance did not completely determine the distribution of hot thermophilic cyanobacteria, but had an important role in isolating this community, possibly leading to allopatric speciation (PAPKE *et al.*, 2003b).

Unique sequences of the thermophilic bacterial genus *Sulfurihydrogenibium* were found in specimens from the thermal springs at Yellowstone National Park (WY, USA), indicating a degree of endemicity in the group and the geographical isolation of the habitats. Despite no direct correlation with geographical distance or the geochemical profile of the springs, it was possible to relate the occurrence of these unique sequences with geographical provinces delineated by pre-historical volcanic eruptions (about 2 million years ago) This shows that historical events, in addition to contemporary environmental factors, can also influence the biogeographical distribution of microorganisms (TAKACS-VESBACH *et al.*, 2008).

In the context of historical events driving microbial biogeography, Bahl *et al. (*2011) showed that pyrosequences of a globally sampled genus of desert cyanobacteria (*Chroococcidiopsis*) presented a strong spatial-temporal pattern of distribution, being strictly divided into groups of hot- and cold-desert specialists. Temporal analyses showed no evidence of significant gene flow among these groups since the formation of the actual continents, reinforcing the importance of past events in microbial biogeography.

Currently, the main challenge in the field of microbial biogeography is to evaluate factors determining geographical distribution, to determine whether historic events (geographic barriers for example), contemporary environmental factors, or a combination of both, drive the drift in microbial diversity (HANSON *et al.*, 2012).

1.2. Taxa-area and distance-decay relationships described for microorganisms.

The taxa-area relationship is one of the most consistent laws in ecology and is well described for macroorganisms. This power law is represented by the equation $\mathbf{S = c\ A^z}$, where **S** is the number of species, **A** is the area sampled, **c** is a constant that is empirically derived from the taxon and the specific location studied, and the exponent **z**, the power law index (i.e. **z**-value), represents the rate of increase in the number of species along an increasing sampling area (graphical slope). When significant, the z-value may present strong evidence for a biogeographical pattern of distribution (BROWN & LOMOLILO, 1998).

Values for the power law exponent have already been described for microorganisms. Interestingly, z-values for microorganisms are often smaller than that for macroorganisms. This result may be attributed to the following factors: (1) larger capacity for dispersion; (2) the lack of a clear “species” resolution; and (3) the use of molecular fingerprinting or sequencing techniques (GREEN *et al.*, 2004; HORNER-DEVINE *et al.*, 2004; BELL *et al.*, 2005). Molecular fingerprinting methods, such as terminal restriction fragment length polymorphisms (T-RFLP) and denaturing gradient gel electrophoresis (DGGE), have proven to be important tools for accessing the diversity of microorganisms in different environments at relatively low cost and with little time consumption (HEAD *et al.*, 1998). However, fingerprinting methods are usually limited as they detect the most common species and therefore underestimate the total diversity in a sample (WOODCOCK *et al.*, 2006). This is mostly because fingerprinting techniques lump different closely related “species” into a single taxonomic unit (often called an operational taxonomical unit, or OTU), and usually ignore rare species. Nevertheless, fingerprinting techniques are still extremely valuable for rapidly comparing the compositions of microbial communities in different environments (MARTINY *et al.*, 2006).

One biogeographical study of microorganisms compared results obtained from a fingerprinting technique (T-RFLP) with those from sequencing. The results were seen to be different mostly with regard to determining which environmental factor influences the differential distribution of the community, but both were able to trace the biogeographical pattern to quite a similar extent (SCHAUER *et al.*, 2010).

Indeed, analysis of gene sequences provides information regarding randomly chosen phylotypes (‘sampling communities’) where the finding of an OTU is proportional to its abundance in the clone library (SCHÜTTE *et al.*, 2008). In contrast, the fingerprinting method T-RFLP screens for all OTUs present above the detection threshold of the method, but does not provide clear taxonomic distinction (DUNBAR *et al.*, 2001)

Another parameter of species distribution through space is the distance-decay relationship, which consists of the decay of similarity between different communities as a function of the distance separating them (NEKOLA & WHITE, 2004), and can also be seen as evidence of a biogeographical pattern. The main difference between the distance-decay approach and the species-area relationship lies in the consideration of the relative abundance of the species compounding the community in the former method, rather than just species richness in the latter. Bell (2010) showed that bacteria living in water-filled tree holes at the same study site displayed a significant distance-decay relationship that was predominantly related to the island type of organization of the habitats in this approach, and Schauer *et al.* (2010) also found a small but significant distance-decay relationship among bacteria from Atlantic Ocean surface sediments, primarily related to geographical distance among the communities and environmental factors.

1.3. Methanogenic Archaea

Among the enormous diversity in microbial groups, methanogenic communities demand special interest, given the fact that methane is one of the most important greenhouse gases (IPCC, 2007). Methane emissions to the atmosphere can originate from either anthropogenic or natural sources. With respect to anthropogenic sources, biogenic sources resulting from human activities, i.e. sewage disposal, enteric fermentation of domestic animals, rice fields, biomass combustion, landfills, and others (KHALIL & SHEARER, 1993), as well as abiogenic sources such as the extraction of fossil fuels, should be taken into account. Natural sources of emissions include wetlands (swamps, lakes, ponds, damps, etc.), termites, ruminants, and the oceans. Among all these sources, the natural sources account for about 30% of the total global methane emissions, totaling approximately 160 $TgCH_4$ per year (IPCC, 2007); and nearly 70% of this total is produced by wetlands and

continental aquatic environments (KHALIL & SHEARER, 1993; WUEBBLES & HAYHOE, 2002).

Tropical wetlands possess great significance for the global budget of gas methane and can contribute around 60% of the total wetland emissions of the planet (LELIEVELD *et al.*, 1998). Lakes within the floodplains of tropical rivers, such as the Amazon (RICHEY *et al.*, 1990), Orinoco (SMITH *et al.*, 2000), and Pantanal (MARANI & ALVALÁ, 2007; BASTVIKEN *et al.*, 2011) rivers, can be a particularly important source of emissions of atmospheric CH_4.

All methane emitted by aquatic continental environments is produced through the anaerobic decomposition of organic matter via acetotrophic methanogenesis, which consists of the conversion of the acetate (a subproduct of organic matter degradation) into methane and carbon dioxide ($CH_3COOH \rightarrow CH_4 + CO_2$), and hydrogenotrophic methanogenesis, which consists of the formation of methane from hydrogen and carbon dioxide ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$). Most methane production occurs through the first process, but depending on the environment and the level of degradation of the organic matter, the ratio between the two processes can vary, and can even be inverted (CONRAD, 1999; CONRAD *et al.*, 2010). In addition, recent studies have shown that in tropical lakes, hydrogenotrophic methanogenesis plays a more important role than acetotrophic methanogenesis (CONRAD *et al.*, 2011).

2. JUSTIFICATION AND OBJECTIVES

Methanogenic Archaea account for approximately all the biogenesis of gas methane around the globe, and methyl coenzyme M reductase is a subunit of the key enzyme of this process. The *mcrA* gene, which codes for this subunit, can therefore be used as a genetic marker for methanogenic groups in specific environments (CONRAD *et al.*, 2008; ANGEL *et al.*, 2012).

Little is known about the geographic distribution of methanogenic archaea. To date, hot desert soil methanogenic archaea have been shown to be widely spread in different parts of the globe, and this pattern of distribution could also be found by reactivation of the cryptic methanogenic process *in vitro* (ANGEL *et al.*, 2012). To the best of our knowledge, there is no description of distance-decay or species-area

relationships for non-extremophilic Archaea in a continuous environment. Given the high ecological stability of methanogenic sediments and soils, with a continuous anaerobic environment and a regular input of organic matter, microbial communities related to the methanogenesis process tend to be stable through time (CONRAD, 2007). Thus, we hypothesized that the change in diversity patterns along different spots of the continuous lake sediment should be strongly correlated to the geographical distance between then.

The Pantanal and Amazonia floodplains are extremely important and diverse biomes, and given the high importance of tropical wetlands in total methane emission by natural sources, these become important environments for the study of the distribution of this particular group of microorganisms (BASTVIKEN *et al.*, 2010; CONRAD *et al.*, 2011)

2.1. General objective

In this study, we targeted 3 different genes and consequently 3 different taxonomical groups, and investigated their distribution in a tropical lake area. The 16S rRNA genes of bacteria and archaea, which are transcribed to generate the structural RNA of the small ribosomal subunit, are universal and strongly conserved genes that are found in all domains of life and are therefore widely used as taxonomic markers (STACKEBRANDT & GOEBEL, 1994). *mcrA* is a functional gene coding for the alpha subunit of methyl-coenzyme M reductase, an enzyme that is essential and characteristic for the biochemical pathway of methanogenesis in Archaea (SPRINGER *et al.*, 1995). Thus, we targeted not only the higher taxonomic levels of prokaryotes, but also the lower taxonomic levels of a particular functional group or ecological guild.

2.2. Specific objectives

2.2.1. To evaluate microbial biogeography

This study attempted to evaluate, using two different approaches (taxa-area and distance-decay relationships), the biogeographical pattern of distribution of 3

different taxonomical groups (Bacteria, Archaea, and Methanogenic Archaea) in the sediment of 2 different tropical floodplains.

2.2.2. To compare the biogeography of different taxonomic groups

We also compared the different values presented by the 3 different taxonomic groups targeted in this work, and attempted to relate these differences with their respective ecological profiles.

2.2.3. To correlate methane production with biogeography

We tried to correlate, in the Amazonia floodplain, the biogeography of the methanogenic Archaea and the rates of methane production in the corresponding sediment, thereby correlating the structure and function of the ecosystem.

3. MATERIALS AND METHODS

We sampled the sediment of 2 floodplains in 2 different regions and biomes of Brazil.

3.1. Sampling and study areas

This study was conducted in two lakes—one located in the Pantanal and another in the Amazon Region (Figure 1).

The Pantanal is the largest floodplain in South America and is periodically flooded by the Paraguay River and its tributaries. The altitude above sea level varies from 80 to 120 m and the total estimated area is around 138.123 km^2. The water flows continuously and thus carries organic material, and during the flood period the sediment spreads all over the plain, constituting the most important source of carbon and nutrients for methanogenic Archaea (MARANI & ALVALÁ, 2007). The climate is hot and wet in the summer, and cold and dry in the winter. The maximum temperature often surpasses 40°C. From May to July, the average temperature drops below 20°C, and the minimum temperature may reach 0°C (GUERRINI, 1978).

In the Pantanal, the study was conducted in a lake (19°02.651′S 57°30.254′W) called *Baía do Arroz* or *Baía Negra* (Figure 1). It is located at the south margin of the Paraguay River and a few kilometers east from the counties of Corumbá and Ladário, close to the Bolivia-Brazil border. *Baía Negra* is a perennial lake; the water depth varies between 2 m and 6 m approximately, and increases or decreases following the flood regime.

The Amazon basin drains a surface area of around 6,000,000 km^2 in the North of South America and is composed of the Amazon River and its large tributaries. Along their courses, these rivers and tributaries are accompanied by large floodplains that cover an area of about 300,000 km^2. Permanent and temporary lakes increase in size and become connected to each other during times of high river discharge (JUNK, 1997; MOREIRA-TURCQ *et al.*, 2004).

In the Amazon Region, the study was conducted at the floodplain of the Grande do Curuai Lake, a complex system with more than 16 interconnected lakes and linked to the Amazon River by 9 watercourses on its southeast side, draining a total area of 3.610 km^2. Such a floodplain is extremely large and shallow and is occupied by vegetation during the low-water period of the year. The total flooded area can vary from 700 km^2 (dry season) to 2.300 km^2 (rainy season). The water level is normally at its lowest point in November and the flooding period is from December to May/July. The Grande do Curuai Lake is a white-water lake, which is characterized by a large amount of suspended sediment (RADAMBRASIL. PROJETO RADAMBRASIL, 1976; MARTINEZ *et al.*, 2003; MOREIRA-TURCQ *et al.*, 2004; MAURICE-BOURGOIN *et al.*, 2005). We sampled one of these lakes in the west extremity of the system in the south margin of the Amazon River (2°4′25.13″S 55°45′23.03″O) (Figure 1).

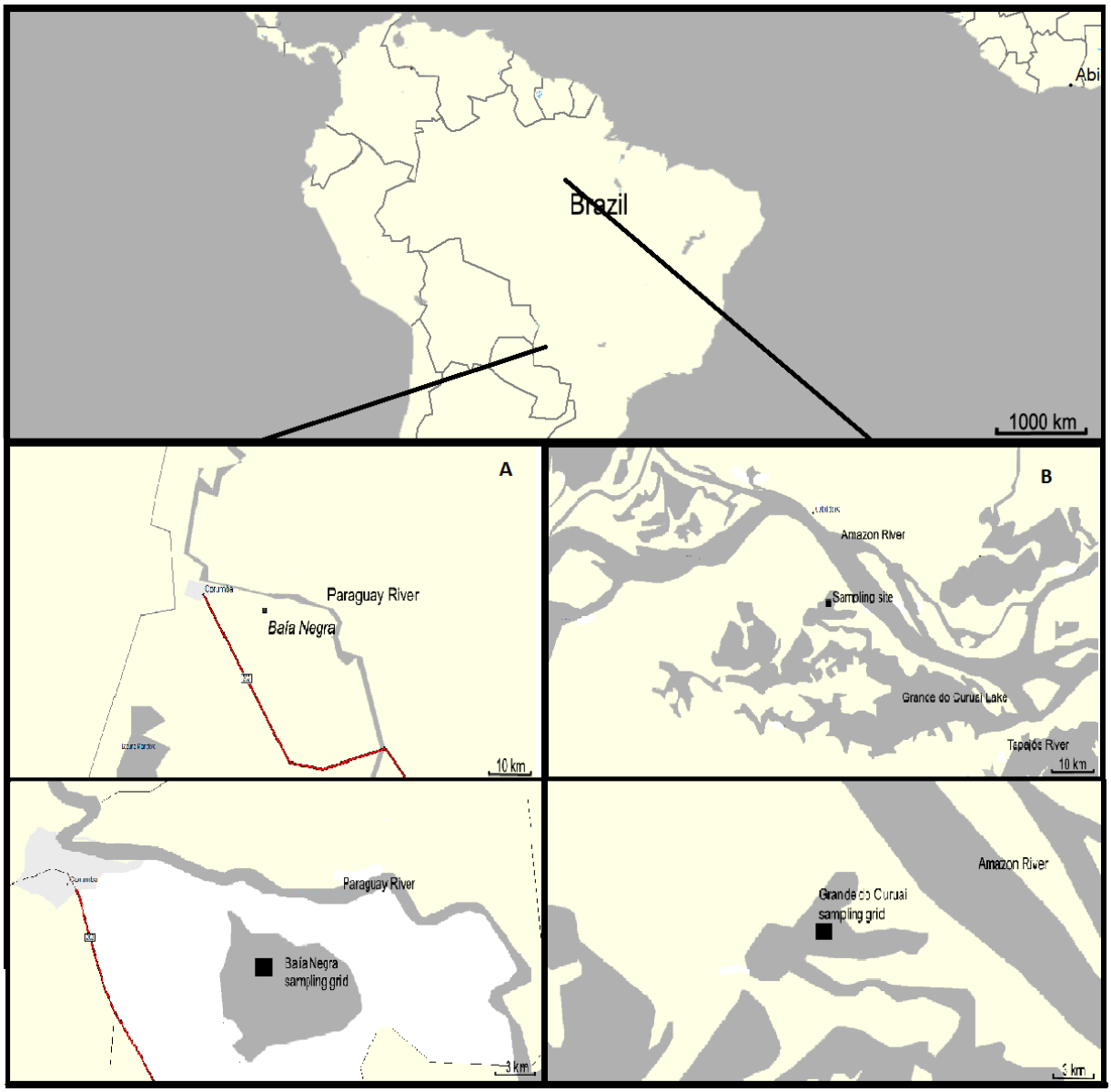


Figure 1 – Sampling sites. Maps of the study locations. The *Negro lake* is represented in **A,** and the Grande do Curuai Lake in **B**. The sampling sites are represented as small black squares. (Maps generated with the MapSource ® Garmin software)

3.2. Community fingerprint profiles

In each lake, 21 sediment cores (diameter, 7cm; height, 120cm) were collected in July 2011. The cores were distributed on a nested square grid with distances between points varying from 0.01m to approximately 1,400m (Figure 2). The 10cm top layer of the sediment was then selected for analysis according to the following procedures: the sediment was homogenized, and 1ml was sampled and frozen for molecular biology analyses. The central core E was subsampled using

polyethylene straws at points 10cm and 1cm apart inside the core area, and 10cm deep.

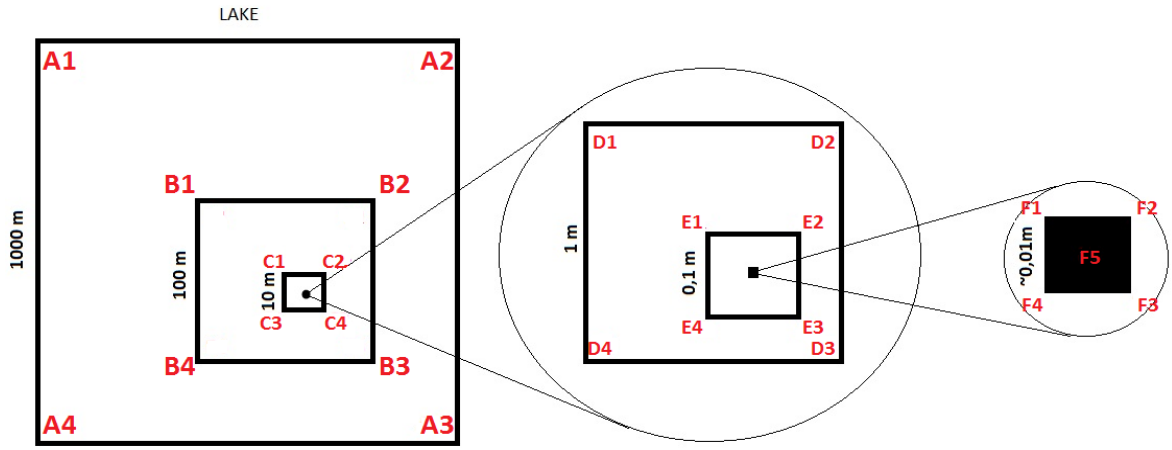


Figure 2 – Sampling grid designed inside the Negro Lake and Grande do Curuai Lake areas; A1–F5 represent the sampling points (Adapted from Horner-Devine, *et al*., 2004).

The frozen sediment samples were thawed, and total DNA was extracted using the FastDNA® SPIN Kit for Soil (MP™ Biomedicals, Carlsbad, CA, USA). Subsequently, the samples were treated 3 times with 5.5 M guanidine thiocyanate as an additional cleaning step during matrix binding. Total DNA was quantified using spectrophotometry. Preparations containing more than 30 ng/µl DNA were considered to be adequate.

Bacterial and archaeal 16S rRNA gene fragments from the *Baía Negra* samples were PCR-amplified using the primer pairs Eub 9/27f (5′-GAG TTT GAT CMT GGC TCA G-3′) and Eub 907/926r (5′-CCG TCA ATT CMT TTR AGT TT-3′), as described by Weisburg *et al*. (1991) for Bacteria, and the primer pairs A109f (5′-ACK GCT CAG TAA CAC GT-3′) and A934b (5′-GTG CTC CCC CGC CAA TTC CT-3′), as described by Grosskopf *et al*. (1998) for archaea. For TRFLP analyses, the forward bacterial and the reverse archaeal primers were labeled with 5-carboxyfluorescein (FAM). Each 50-µl PCR reaction volume contained 1× GoTaq®Flexi Green Buffer (Promega®, Madison, WI, USA); 1.5 mM $MgCl_2$ (Promega®); 200 µM dNTP Mix (Fermentas®, Waltham, MA, USA); 0.33 µM of each primer described (Sigma™, St. Louis, MO, USA); 1 U GoTaq™Flexi DNA Polymerase (Promega®), and 10 µg of bovine serum albumin (BSA) (Roche®). Diluted total DNA extract was added as the

template (1 µl). The reaction was initiated with a denaturation step (94°C for 3 min), followed by 24–28 cycles of denaturation (94°C for 45 sec), annealing (52°C for 45 sec), and extension (72°C for 80 sec), and a final extension step (72°C for 5 min).

For the amplification of the *mcrA* gene in both lakes we used the primer pair MCRf (5′-TAY GAY CAR ATH TGG YT-3′) and MCRb (5′-ACR TTC ATN GCR TAR TT-3′) as described by Springer *et al*. (1995), the forward primer being labeled with FAM for T-RFLP analyses. Each 50-µl PCR reaction volume contained 1× MasterAmp™ PCR PreMix B (Biozym®), 0.33 µM of each primer described, 1 U GoTaq®Flexi DNA Polymerase (Promega®), 10 µg BSA (Roche®, Basel, Switzerland), and 1 µl of DNA template. The reaction started with an initial denaturation step (94°C for 3 min), followed by 32 cycles of denaturation (94°C for 45 sec), annealing (50°C for 45 sec), and extension (72°C for 90 sec), and a final extension step (72°C for 5 min).

The PCR quality was controlled using agarose gel (1.5%) electrophoresis. The DNA was then purified using the GenElute™ PCR Clean-Up Kit (Sigma®) following the manufacturer´s instructions, and stored at -20°C.

For T-RFLP, the PCR product was digested using the following restriction enzymes: *Msp*I incubated overnight at 37°C for bacterial 16S rRNA genes, *Taq*I incubated for 3 h at 65°C for archaeal 16S rRNA genes, and *Sau*96 incubated for 3 h at 37°C for *mcrA*. After incubation, the digested products were once again cleaned with the SigmaSpin™ Sequencing Reaction Clean-Up, Post-Reaction Purification Columns (Sigma®). The samples were denatured at 94°C for 2 min and loaded onto an ABI 3100 automated gene sequencer (Applied Biosystems, Foster City, CA) for separation of the TRFs. T-RFLP data were retrieved by comparison with an internal standard using GeneScan 3.71 software (Applied Biosystems).

3.3. Taxa-area and distance-decay relationships

The T-RFLP profiles were analyzed and standardized as described in Dunbar *et al*. (2001) resulting in T-RFs 60 to 855 base pairs (bp) in size, each representing more than 1% of the total fluorescence of that sample.

We used the 25 resulting profiles for pair-wise calculation of the Bray-Curtis dissimilarity indices (BRAY & CURTIS, 1957) and the Sorensen similarity indices, resulting in 300 different pairs for each targeted gene. A simple linear regression of the log-transformed data of the Bray-Curtis indices, plotted against the distances between the sediment samples from which the pairs originated, was used to estimate the slope of the distance-decay relationship (NEKOLA & WHITE, 2004).

The same log-transformed linear regression was used to calculate the slope of the Sorensen indices plotted against the distance between points. The resultant similarity slope was used to calculate the z-value of the taxa-area relationship with the formula $\mathbf{\log(S_S) = constant - 2}z\ \mathbf{\log(D)}$, where S_S is the pair-wise similarity between communities and D is the distance between the two samples used in the distance-decay approach, and the z-value is determined as the – (**regression coefficient)/2** as described by Harte *et al*. (1999).

In order to avoid randomization patterns that could influence the results, the same calculations were performed utilizing distances smaller than 200 m and 20 m, and the results were similar to those of the complete data set.

3.4. Methane production

At the Curuai lake, the sediment was also sampled for methane production determination. Approximately 10-ml aliquots of the sampled sediment were incubated at 25°C in sterile test tubes (in a 27-ml volume) sealed with rubber stoppers, and flushed with nitrogen. The samples were divided into 2 groups: one was the control group and the other had fluoromethane (CH_3F), an inhibitor of the acetotrophic pathway of methanogenesis as described by Conrad (2007), added to it, allowing the determination of the prevalence of hydrogenotrophic and acetotrophic methanogenesis. Periodically (usually every 2–3 days), 250-µl headspace gas

samples were taken with a chromatography syringe and the methane and CO_2 concentrations were measured by gas chromatography (flame ionization detection).

Methane production rates with different treatments were obtained by dividing the total concentration of methane at the end of the log phase of methane production by the total hours of incubation and by the total dry weight of the sediment, as described by Conrad *et al*. (2011).

Thus, we calculated the arithmetic mean of the rates from each of the squares of different sizes that composed the sampling grid, representing different geographical distances (A, B, C, and D) (Figure 2). We compared these means in terms of their absolute values (two-way ANOVA) and their coefficient of variation (CV).

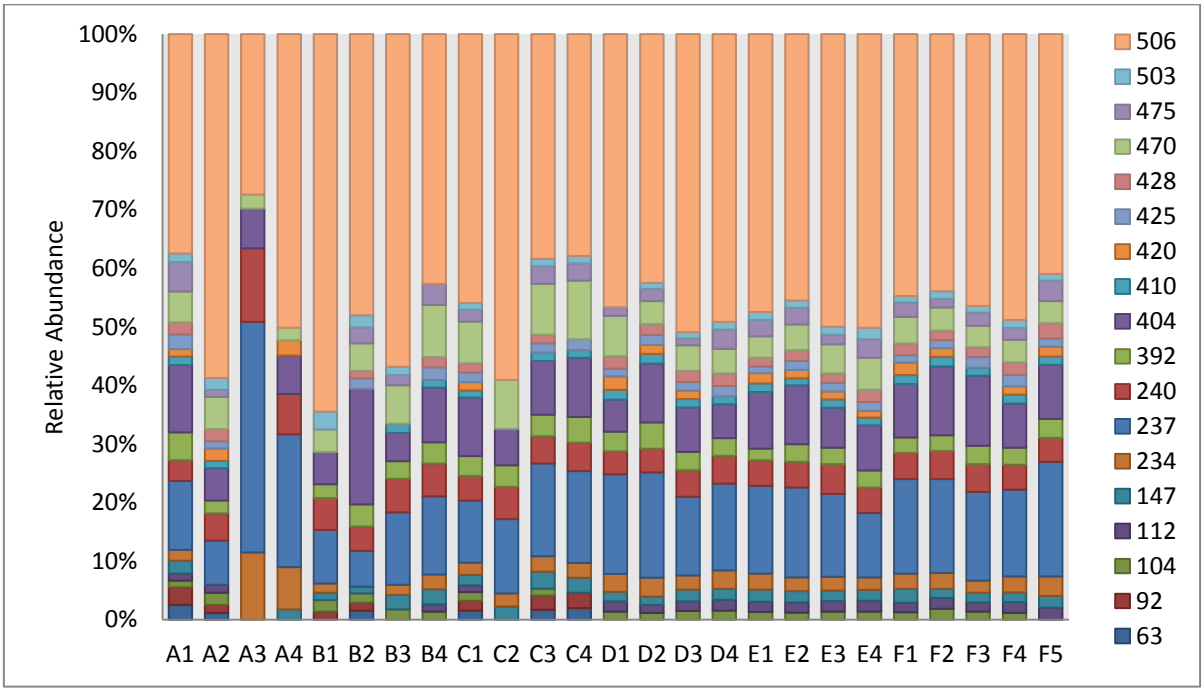
4. RESULTS

4.1. *Baía Negra* – Pantanal

4.1.1. Community profile

The T-RFLP profiles were similar between sampling points for every analyzed gene (Figure 3). Analysis of the *mcrA* T-RFLP profile resulted in a total richness of 18 different OTUs of methanogens through the lake area (Figure 3A). The OTU with 506 bp showed the highest relative abundance. For the archaeal 16S rRNA T-RFLP profile, the total richness was 22 different OTUs (Figure 3B) with none of them showing dominance over the others. The bacterial 16S rRNA gene T-RFLP profiles showed the largest OTU richness, with a total of 37 different OTUs found in the profiles across the lake area, showing a high diversity of dominant groups (Figure 3C). In a literature-based comparison, it was possible to relate some of the observed *mcrA* OTUs founded with previously described ones, keeping in mind that such affiliations can only be tentative (Table 1). These affiliations with phylogenetic groups are based on data from literature that used the same primers and restriction enzymes for T-RFLP. All the affiliations are only tentative, and can only be interpreted as the probable main groups of the lake sediment community.

A


Relative Abundance
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90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
A1 A2 A3 A4 B1 B2 B3 B4 C1 C2 C3 C4 D1 D2 D3 D4 E1 E2 E3 E4 F1 F2 F3 F4 F5
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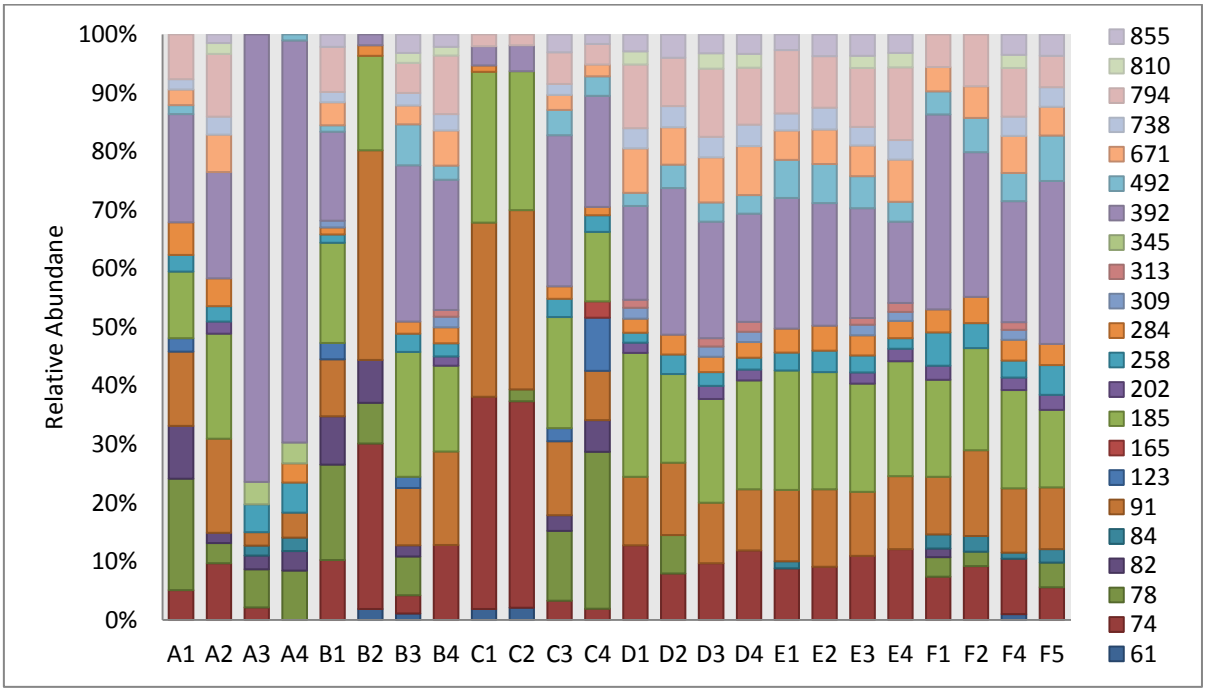
B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855
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C

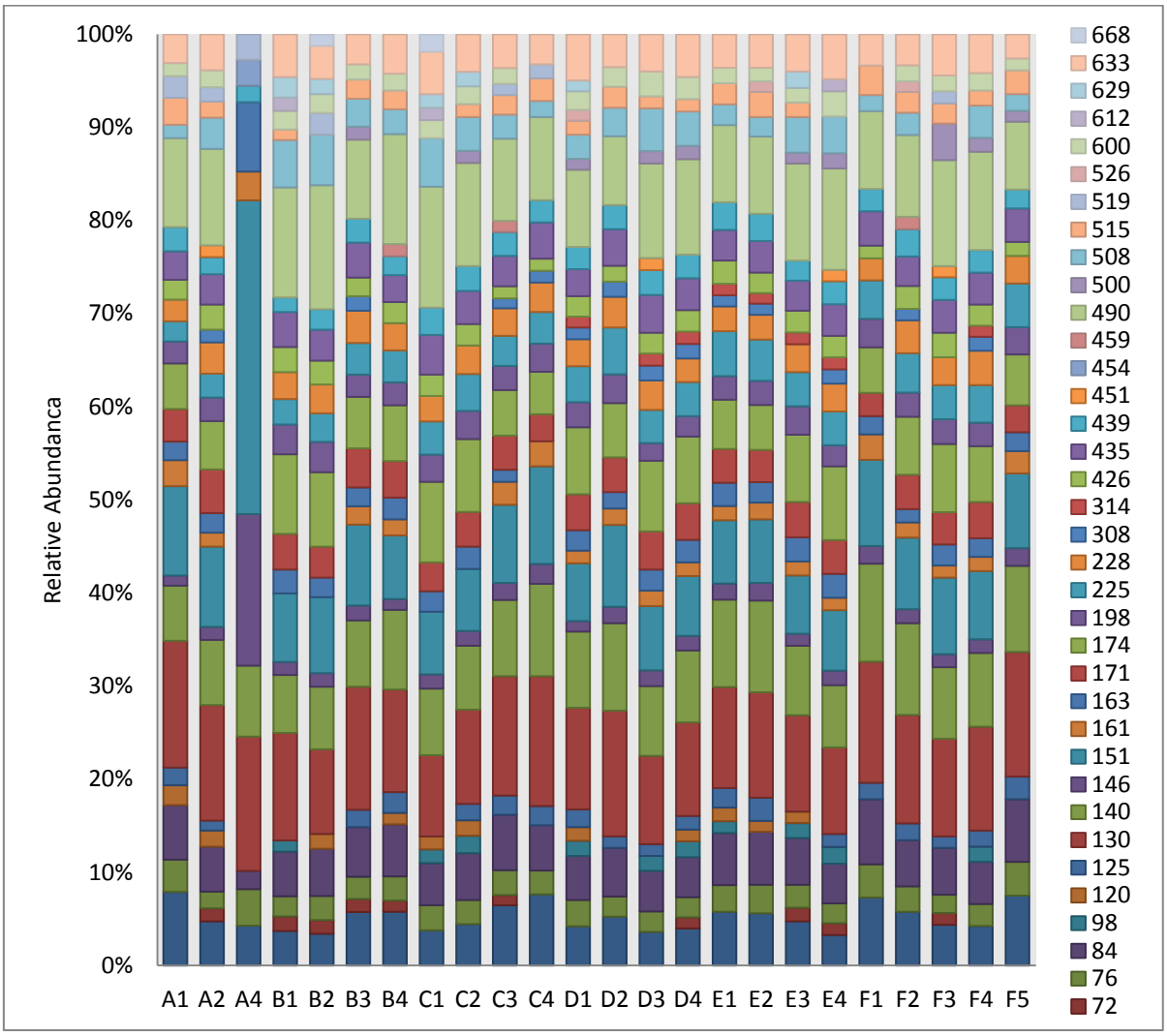

Figure 3 – T-RFLP profiles of three different genes studied from sediment samples obtained inside the area of the *Baía Negra*. A1–F5 represent the sampling sites (see Figure 2 for more details), the bar size represents the relative abundance of each of the TRFs, which are defined by their size in base pairs (bp). (A) *mcrA*, (B) archaeal 16S rRNA gene, and (C) bacterial 16S rRNA gene.

Table 1 – Tentative genetic affiliations of the OTUs – For the *mcrA* gene in the Negro Lake area, genetic affiliations with different phylogenetic groups have been described in the literature: some of these are shown here.

Gene	TRF Size (bp)	Tentative Phylogenetic Affiliation*	Reference
mcrA	147	Methanosaetaceae	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008; LUEDERS *et al.*, 2001; RAMAKRISHNAN, 2001)
	234	Methanocellales	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008; LUEDERS *et al.*, 2001; RAMAKRISHNAN, 2001)
	237	Methanocellales	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008; LUEDERS *et al.*, 2001; RAMAKRISHNAN, 2001)
	392	Methanosarcinaceae	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008; KEMNITZ *et al.*, 2004; LUEDERS *et al.*, 2001; RAMAKRISHNAN, 2001)
	404	Methanobacteriaceae	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008; KEMNITZ *et al.*, 2004; LUEDERS *et al.*, 2001)
	420	Methanosaetaceae	(KEMNITZ *et al.*, 2004; LUEDERS *et al.*, 2001)
	470	Methanobacteriaceae	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008; LUEDERS *et al.*, 2001; RAMAKRISHNAN, 2001)

All the three T-RFLP profiles showed a significant distance-decay relationship **(Y)** (Figure 4). The *mcrA* gene showed the largest slope of 0.14 ($P < 0.0001$, $R^2 = 0.56$), followed by the archaeal 16S rRNA gene with a slope of 0.11 ($P < 0.0001$, $R^2 = 0.26$), while the slope for the bacterial 16S rRNA gene was 0.067 ($P < 0.0001$, $R^2 = 0.17$).

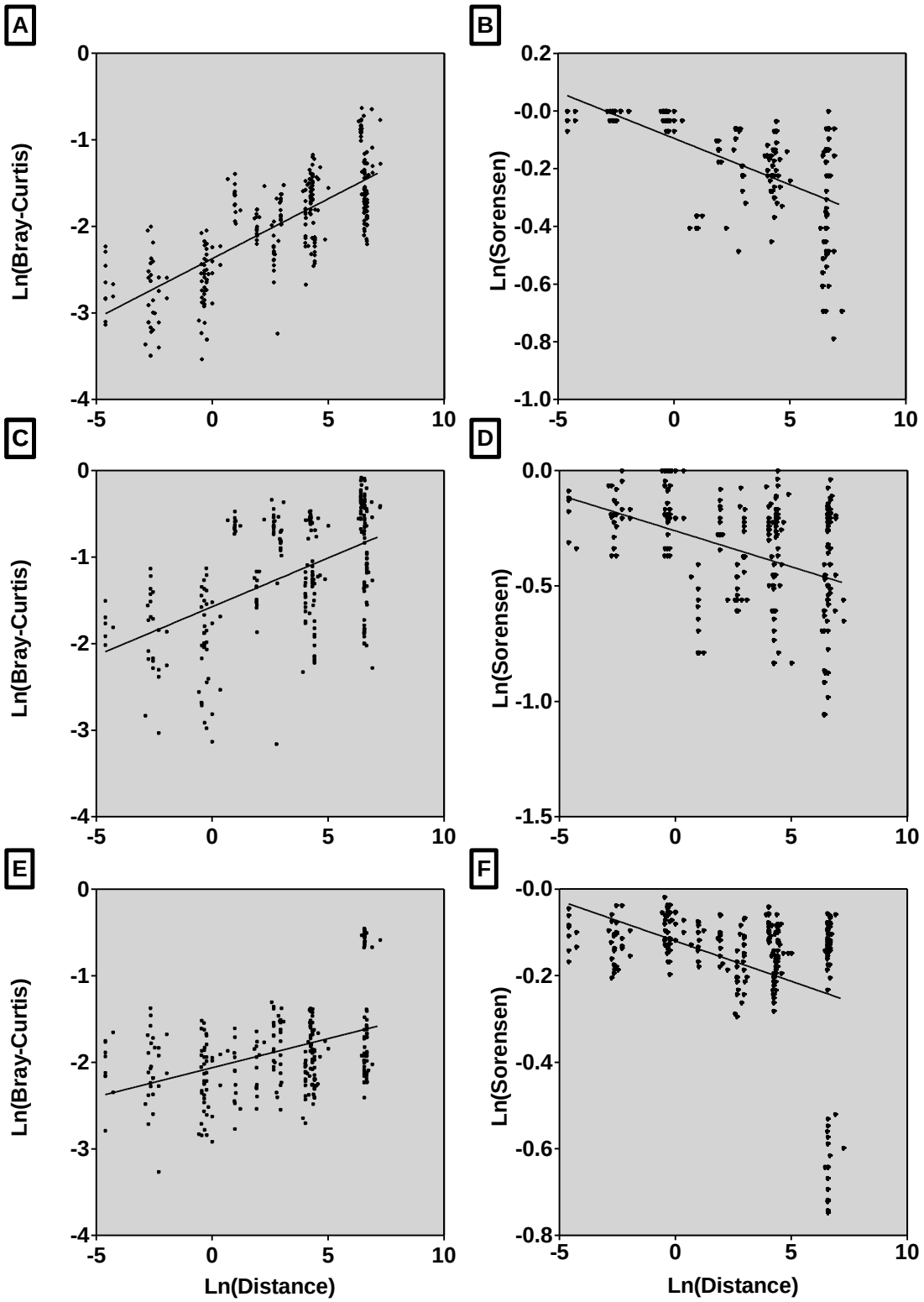

Figure 4 – Log-transformed distance-decay (Bray-Curtis) and taxa-area (Sorensen) relationships for the 3 different genes. (A and B) *mcrA*; (C and D) archaeal 16S rRNA gene and (E and F) bacterial 16S rRNA gene.

The regression coefficient based on the Sorensen similarity indices calculated for all the 3 genes also showed significant values (Figure 4) of -0.032 ($P < 0.0001$, $R^2 = 0.33$) for *mcrA*, -0.031 ($P < 0.0001$, $R^2 = 0.23$) for archaeal and -0.018 ($P < 0.0001$, $R^2 = 0.14$) for bacterial 16S rRNA genes. However, the calculated z-values were small, indicating relatively low taxa-area relationships (Table 2).

Table 2 – Distance-decay regression coefficients based on the Bray-Curtis dissimilarity index **(Y)**, Regression coefficient based on the Sorensen Similarity index, and the exponent **z** calculated by the distance-decay approach values, for each of the target genes, calculated by –(regression coefficient)/2

Gene	***Distance-decay (Y)***	***Sorensen Regression Coefficient***	***(z)***
mcrA	0.14	-0.032	0.016
Archaea 16S	0.11	-0.031	0.0155
Bacteria 16S	0.067	-0.018	0.009

4.2. Grande do Curuai Lake – Amazon

4.2.1. Community profile

The complete T-RFLP profile of every single site sampled in the lake is shown in Figure **5.** A total of 27 different OTUs were described by the T-RFLP profiles of the *mcrA* gene, and the 506-bp fragment showed relatively greater abundance at all sampling sites. Some OTUs were highly abundant, such the 421-bp and 241-bp fragments, but their relative abundance was not very high in the samples analyzed. The sampling location A1 presented the largest absolute richness (16 OTUs), while the sampling location B2 presented the lowest (7 OTUs). Tentative phylogenetic affiliations of some of the OTUs found in this study, based on data from literature, are presented in Table 3.

Table 3 – Tentative genetic affiliation of the OTUs – For the mcrA gene found in the Grande do Curuai Lake, genetic affiliations with different phylogenetic groups have been described in the literature; some of these are shown here.

Gene	TRF Size (bp)	Phylogenetic affiliation	Reference
mcrA	**147**	Methanosaetaceae	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008; LUEDERS *et al.*, 2001; RAMAKRISHNAN, 2001)
	237	Methanocellales	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008; LUEDERS *et al.*, 2001; RAMAKRISHNAN, 2001)
	241	Methanosaetaceae	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008)
	392	Methanosarcinaceae	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008; KEMNITZ *et al.*, 2004; LUEDERS *et al.*, 2001; RAMAKRISHNAN, 2001)
	404	Methanobacteriaceae	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008; KEMNITZ *et al.*, 2004; LUEDERS *et al.*, 2001)
	407	Methanobacteriaceae	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008; LUEDERS *et al.*, 2001; RAMAKRISHNAN, 2001)
	421	Methanosaetaceae	(KEMNITZ *et al.*, 2004; LUEDERS *et al.*, 2001)
	425	*Methanosarcina* spp. mcrA	(KEMNITZ *et al.*, 2004; LUEDERS *et al.*, 2001; RAMAKRISHNAN, 2001)
	428	Methanomicrobiaceae	(RAMAKRISHNAN, 2001)
	470	Methanobacteriaceae	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008; LUEDERS *et al.*, 2001; RAMAKRISHNAN, 2001)
	504	Methanobacteriaceae	(CHIN *et al.*, 2004; CONRAD *et al.*, 2008; KEMNITZ *et al.*, 2004; LUEDERS *et al.*, 2001; RAMAKRISHNAN, 2001)

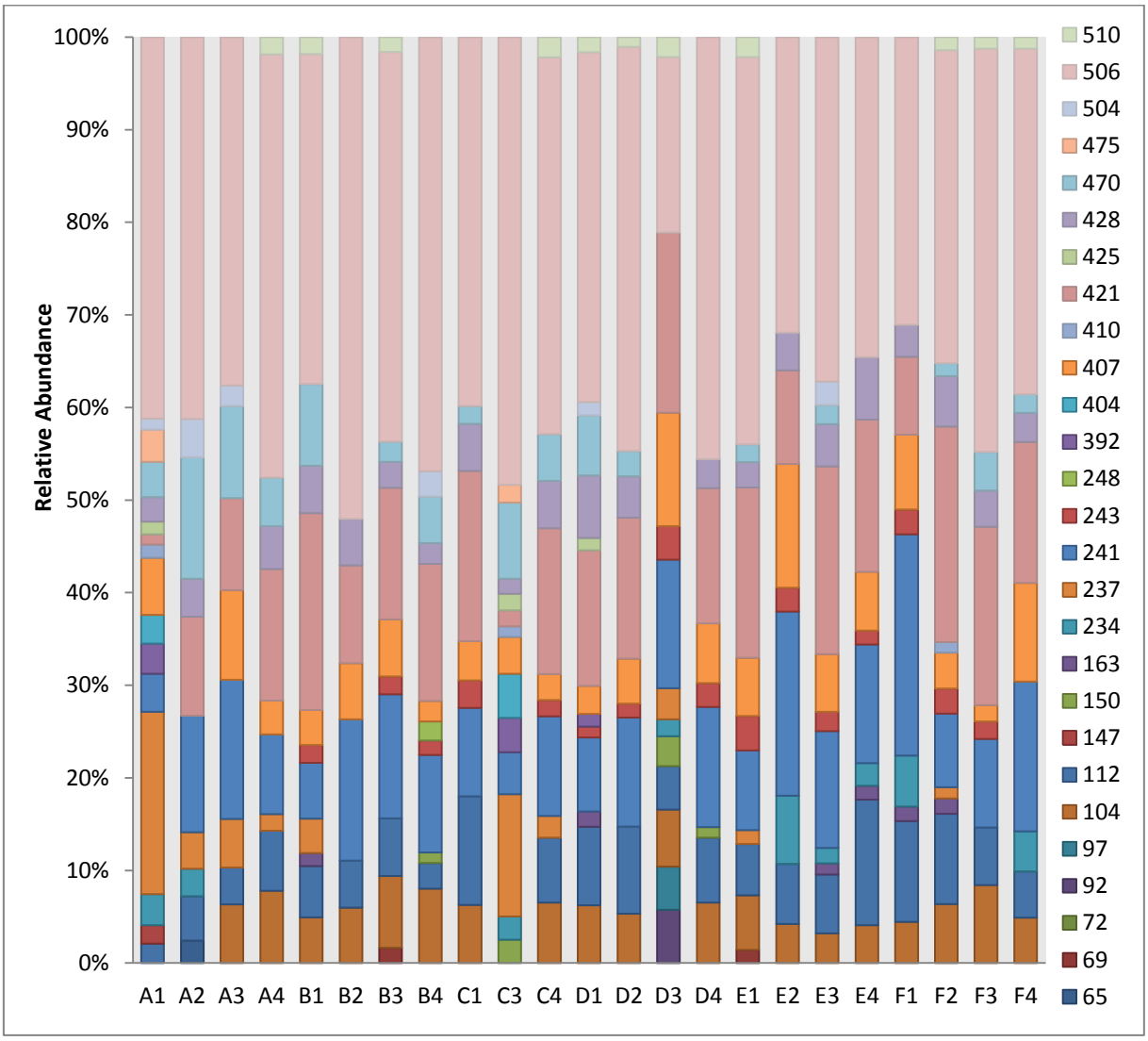

Figure 5 – T-RFLP profiles of the mcrA gene in sediment samples obtained inside the area of the Grande do Curuai Lake. A1–F4 represent the sampling points (see Figure 2 for more details), and the bar size represents the relative abundance of each of the TRFs, which are defined by their size in base pairs (bp).

The Grande do Curuai Lake exhibited a taxa-area relationship and a distance-decay relationship in the methanogenic Archaea community. The Bray-Curtis dissimilarity indices plotted against the distance between the points (Figure 6A), which directly represents the distance-decay relationship of the community, also showed a significant regression coefficient (Y): 0.03103 ± 0.009267 ($P = 0.009$, $r^2 = 0.04277$).

The linear regression slope of the calculated Sorensen Indices plotted by the distance between the sampling points (Figure 6B) showed a regression coefficient of -0.01814 ± 0.003271 ($P < 0.001$, $r^2 = 0.1092$), resulting in a z-value of **0.009**.

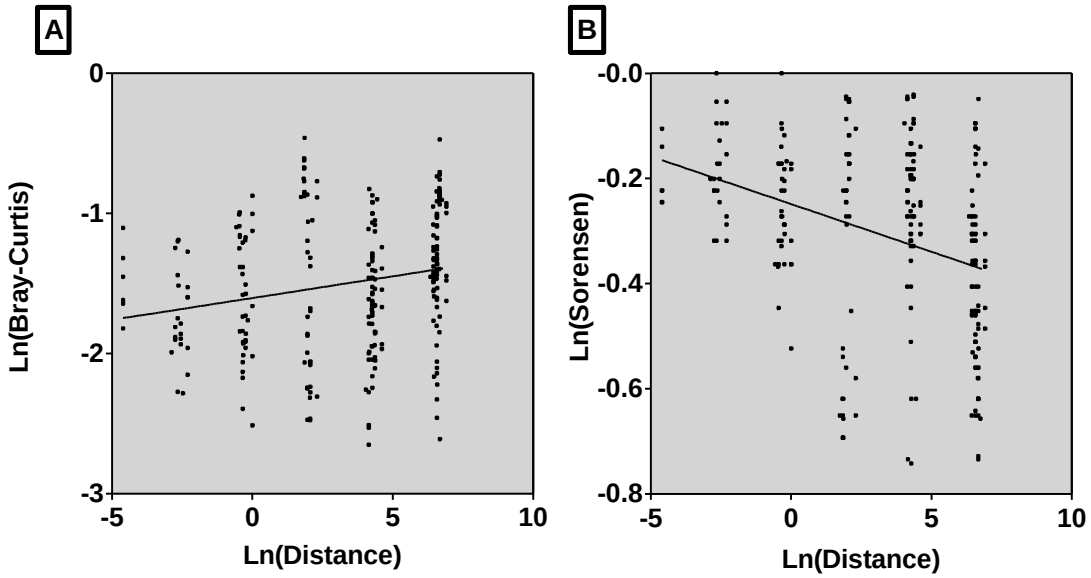

Figure 6 – Log-transformed distance-decay (Bray-Curtis) (A) and taxa-area (Sorensen) (B) relationships for the mcrA gene.

4.2.2. Methane production

The sediment methane production rates are presented in Figure 7. Two-factor analyses (two-way ANOVA) of the different sets of samples (A, B, C, and D) revealed that geographical distance had no significant effect on methane production rate or on the interaction of fluoromethane treatment with geographical distance. However, the coefficient of variation (CV) of the methanogenesis rate increased with distance. Samples that were apart from each other by 1 km showed high CV values: up to 80% for total methane production rates, and over 100% for hydrogenotrophic methanogenesis (indicated by CH_3F treatment). The closest samples showed lower CV values, all below 40%. It is important to note as well that, for any distance analyzed, the inhibited rates showed higher CV values than total rates.

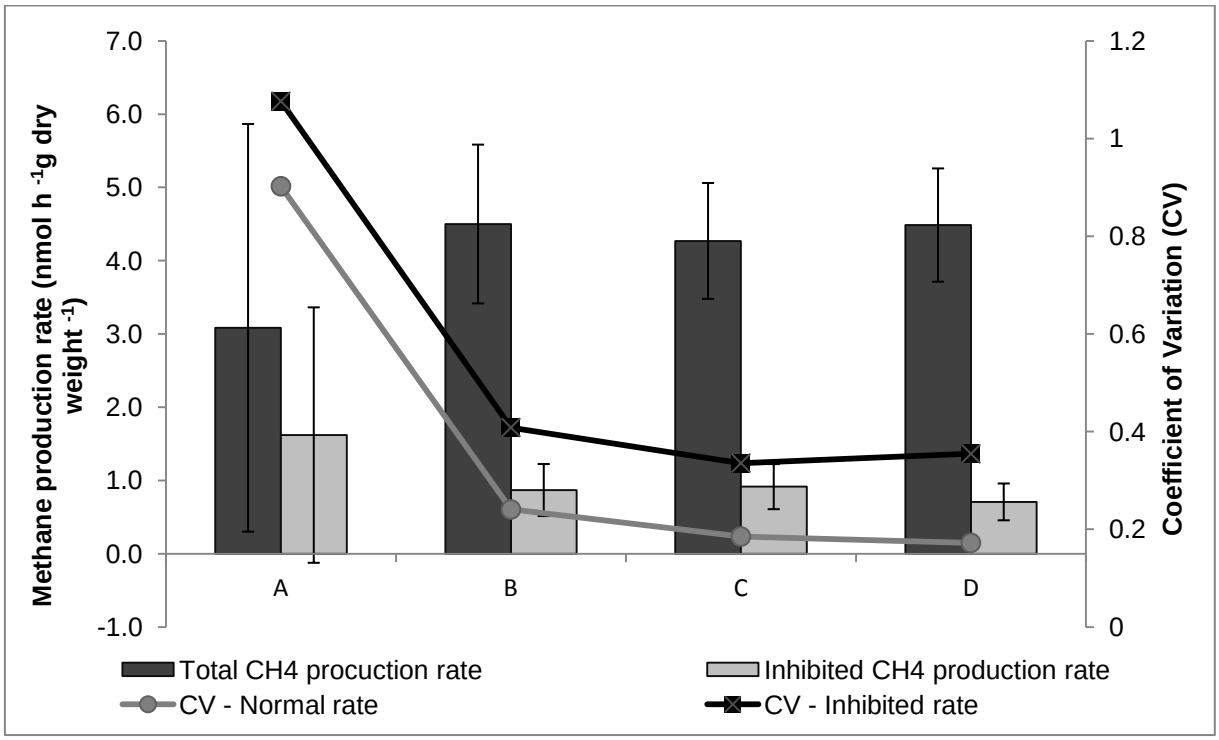

Figure 7 –Methane production rates of the 4 different squares gridded inside the Grande do Curuai lake area. A represents the 1000-m grid, B, the 100-m grid, C the 10-m grid and D the 1-m grid (mean ± SD). Light and dark gray bars denote data obtained with and without addition of inhibitor, respectively. The lines on the secondary axis represent the Coefficient of Variation of methane production.

Treatment with the inhibitor exerted a significant effect on the rate of methane production. Methane production was dominated by hydrogenotrophic methanogenesis, representing ~80% of total methanogenesis.

Table 4 – Two-way ANOVA analysis of methane production rates and distances

Factor	Degrees of Freedom	Sum of squares	*F*	*P*
Treatment	1	74.74	44.42	<0.0001
Distance	3	0.49	0.1	0.9606
Interaction	3	6.95	1.38	0.2738
Error	24	40.38		

5. DISCUSSION

Our study investigated the biogeographical distribution profile of the microbial community in lake sediment, which provides continuous and stable ecological conditions for methanogenic microbial communities. The conclusion is based on the distance-decay relationship of the operational taxonomic units of three different microbial genes. All dominant operational taxa (more than 10% relative abundance) from the 3 genes analyzed in this study were observed at all the sampling points within the lake. Nevertheless, a small but significant taxa-area relationship could be observed. Such a relationship was expected given the high capacity of the targeted microorganisms for dispersion. However, the similarity distance-decay index, which also takes into consideration the relative abundance of the groups, shows that the richness is not homogeneously distributed within the entire lake area. We therefore conclude that the most distant samples were most different from each other.

Studies of bacterioplankton distribution in lakes have shown similar patterns between different and unconnected lakes in distant geographic regions, but with variations in their relative abundance (LINDSTRÖM & LESKINEN, 2002). On the other hand, distance dissimilarities in bacterioplankton communities within one lake were weaker than among different lakes in North America, and mostly influenced by different water regimes and partial geographic isolation (YANNARELL & TRIPLETT, 2004). Some studies in saline lakes in China, Mongolia, and Argentina showed that bacterial biogeography in these environments was based on contemporary environmental factors (Na^+, CO_3^{2-}, and HCO_3^- ion concentrations, pH and temperature) and geographic distance, whereas archaeal biogeography was mainly influenced only by environmental factors (PAGALING *et al.*, 2009). In this study, we showed that biogeographic patterns of microbial communities are also observed when there are no geographic barriers or extreme environmental factors limiting the dispersion process, thus corroborating our initial hypothesis.

The tentative phylogenetic affiliation for the *mcrA* gene (Tables 1 and 3) shows that despite the large diversity of OTUs found in sediment samples from both lakes, efficient taxonomic resolution is still lacking (expected for T-RFLP). When some affiliation exists, it can vary in level from genus to order. Therefore, more extensive

work on the database for this functional gene and its respective TRFs are crucial for a better understanding of the diversity profile for this kind of environment (DUNBAR *et al.*, 2001).

The **z**-values and the distance-decay regression coefficients **(Y)** observed in this study are lower than others previously observed in the literature (GREEN *et al.*, 2004; HORNER-DEVINE *et al.*, 2004; BELL *et al.*, 2005; NOGUEZ *et al.*, 2005; VAN DER GAST *et al.*, 2005; FIERER & JACKSON, 2006), but are the first ones described for bacteria, archaea, and methanogenic archaea in a single continuous environment. The existence of significant z-values shows that all these microbial groups have biogeographic distribution. The low z-values observed were expected given the use of a fingerprinting technique to access the microbial diversity of the target environments, and not a high-resolution ribotyping technique. At an OTU resolution of 95% sequence similarity of the 16S rRNA gene, Horner-Devine *et al*. (2004) described similar z-values for bacteria (z = 0.019) and beta-proteobacteria (z = 0.008) in salt marshes. At higher resolutions, the **z**-values were higher (0.04 for bacteria and 0.019 for beta-proteobacteria at 99% sequence similarity). It is interesting to note that the lower values described in the referenced study were presented by the lower taxonomical levels reached, whereas in the present study we observed the opposite. One possible explanation for this discrepancy could be because we used a functional gene to reach the diversity of the lower taxonomical level. **z**-values reported for other microorganisms are displayed in Table 5.

Table 5 – **z**-values previously described for microorganisms. The numbers 99% or 95% represent the taxonomic resolution applied to define the OTUs (sequence similarity). *not significantly different from zero

Microbial Community	*z-value*	Diversity Access	Reference
Desert Soil Fungi	0.074	fingerprint (ARISA)	(GREEN *et al.*, 2004)
Salt-Marsh Bacteria (99% OTU)	0.04	sequencing	(HORNER-DEVINE *et al.*, 2004)
Salt-Marsh ß-proteobacteria (99% OTU)	0.019	sequencing	(HORNER-DEVINE *et al.*, 2004)
Salt-marsh Bacteria (95% OTU)	0.019	sequencing	(HORNER-DEVINE *et al.*, 2004)

Salt-Marsh ß-proteobacteria (95% OTU)	0.008	sequencing	(HORNER-DEVINE *et al.*, 2004)
Water-filled tree holes Bacteria	0.26	fingerprint (DGGE)	(BELL *et al.*, 2005)
Metal-cutting fluid sump Bacteria	0.26-0.29	fingerprint (DGGE)	(VAN DER GAST *et al.*, 2005)
Soil Bacteria	0.03	fingerprint (T-RFLP)	(FIERER; JACKSON, 2006)
Tropical Forest Soil Bacteria	0.42 and 0.47	fingerprint (T-RFLP)	(NOGUEZ *et al.*, 2005)
Freshwater lake sediment Bacteria	0.009	fingerprint (T-RFLP)	**this study**
Freshwater lake sediment Archaea	0.0155	fingerprint (T-RFLP)	**this study**
Freshwater lake sediment methanogenic Archaea	0.016	fingerprint (T-RFLP)	**this study**
Freshwater lake sediment methanogenic Archaea	0.009	fingerprint (T-RFLP)	**this study**

The differences between the z-values and distance-decay values **(Y)** described for the 3 different genes, that is, 3 different groups of microorganisms, may be explained by 2 hypotheses. The first one is that bacteria (16S rRNA) and archaea (16S rRNA) have in general a higher capacity for dispersal than methanogenic archaea (*mcrA*). By sampling the first 10 cm of the sediment, we were able to access different communities living at different depths at the same time. We assume that the methanogenics were preferably located in the deeper sediment layers, since their activity is inhibited by the presence of other electron acceptors like oxygen, nitrate, iron, and sulfate, potentially present in the surface layers of the sediment. On the other hand, Bacteria and archaea were in general not restricted to deeper sediment layers (LOVLEY & KLUG, 1986; FALZ *et al.*, 1999; CHAN *et al.*, 2005). Archaea, and more significantly bacteria showed weaker species-area and distance-decay relationships than the methanogens (*mcrA*). The richness of archaea and bacteria OTUs was well distributed throughout the lake area, demonstrating the high diversity of microhabitats that can be exploited by these groups, and suggesting that the ability of dispersion seems not to be compromised at smaller scales.

The second hypothesis is based on the taxonomic sensitivity of the T-RFLP method. OTUs can represent a large variety of different taxonomic groups. The universal primers targeting 16S rRNA genes do not represent the entire set of conceivable species, which include many more than the OTUs derived from T-RFLP. Hence, the total diversity of the community is underestimated by T-RFLP (LIU *et al.*, 1997). Another study targeting 16S rRNA genes of the bacterioplankton of temperate lakes showed that the sequence similarity within a single OTU varied from 73 to 100%. However, sequence homology of 97% is generally used to define bacterial species (EILER & BERTILSSON, 2004). When utilizing a functional gene such as *mcrA*, the probability of reaching lower taxonomic levels is higher, because translated genes show diminished conservation and can present higher codon variability, and thus a higher chance of being differentiated by terminal fragment size methods (MARSH, 1999). This may explain why the z-values were higher in T-RFLP analysis of *mcrA* than that of 16S rRNA genes, but it does not explain why the archaeal distance-decay and **z**-values were higher than those of the bacteria, as both of them targeted 16S rRNA genes.

Methane production rates at different sampling stations of the Grande do Curuai lake indicated that the variability increases with distance. The most distant samples showed the more discrepant rates of methanogenesis, in agreement with the significant changes presented by the methanogenic community structures (Figure 8). The maximum concentration of methane ranged from less than 100 to almost 1000 $nmol.ml^{-1}$ on sampling sites 1000 m apart. On the other hand, samples located 1 m apart showed almost identical concentrations of methane during the entire incubation time.

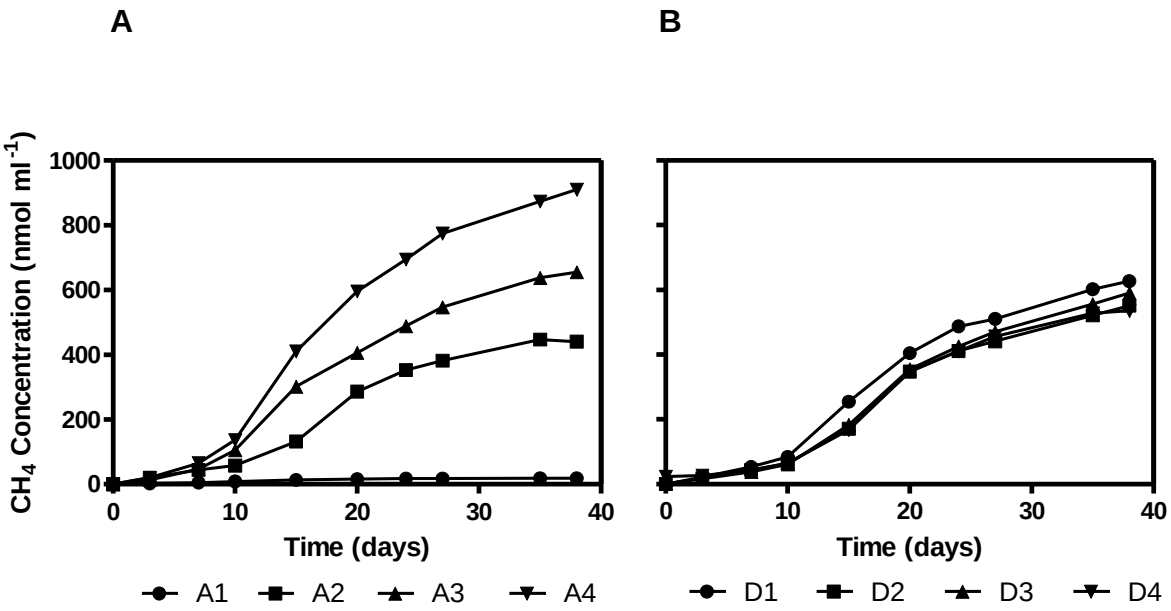

Figure 8 – Example of the discrepancy among methanogenesis rates in the different squares of the grid inside the Grande do Curuai lake area. Panel **(A)** represents the sampling sites 1000 m apart (A grid, see Figure 2) and **(B)** the sampling sites 1 m apart (D grid).

6. CONCLUSION

We conclude that there is a significant geographic distribution pattern for methanogenic archaea, archaea, and even bacteria in a continuous stable environment, i.e. the sediment of two tropical lakes. We also observed the importance of relative abundance as an ecological factor when trying to understand the dynamics of the spatial distribution of microbial communities.

The structure of the communities through space and their ecological role indicates that there is an intra-ecosystemic relationship between diversity and function, related to geographical distance. It means that the more distant the microbial communities, the more they differ in terms of structural (diversity) and functional (methanogenesis) aspects.

Given this scenario, our future studies in the field will focus on unraveling the factors that are driving this type of spatial distribution. Either contemporary environmental or historical (recent and ancient) factors could directly influence these communities, and limiting population dispersion. For now, sorting a high amount of ecological variables, and testing their influence on microbial community structure and

their differential distribution throughout geographical space, should give us a better understanding of the ecological background of methanogenics and the biogeography of microorganisms.

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