Aquatic Science and Technology
ISSN 2168-9148
2013, Vol. 1, No. 1

Changes in Microbial Communities along a Water Column in an Amazonian Flooded Area

Diego A Graças (Corresponding author)

Laboratório de Polimorfismo de DNA, Universidade Federal do Pará

Instituto de Ciências Biológicas, Postal Code 479, Pará State, Brazil

Tel: 55-91-3201-8416 E-mail: diego.a87@gmail.com

Ederson C Jesus

Laboratório de Polimorfismo de DNA, Universidade Federal do Pará

Instituto de Ciências Biológicas, Postal Code 479, Pará State, Brazil

Centro Nacional de Pesquisa de Agrobiologia

BR465, km 7, Seropédica, Rio de Janeiro 23890-000, Brazil

Luciano C F Filho

Laboratório de Polimorfismo de DNA, Universidade Federal do Pará

Instituto de Ciências Biológicas, Postal Code 479, Pará State, Brazil

Rubens Ghillardi Jr.

Centrais Elétricas do Norte do Brasil S.A - Eletronorte - SCN Quadra 06 Conj. A, Blocos B e C, Entrada Norte 2, Asa Norte - Cep 70.716-901 - Brasília/DF

Maria SR Barbosa

Laboratório de Polimorfismo de DNA, Universidade Federal do Pará

Instituto de Ciências Biológicas, Postal Code 479, Pará State, Brazil

Rommel Thiago Jucá Ramos

Laboratório de Polimorfismo de DNA, Universidade Federal do Pará

Instituto de Ciências Biológicas, Postal Code 479, Pará State, Brazil

Tiago F Leão

Laboratório de Polimorfismo de DNA, Universidade Federal do Pará

Instituto de Ciências Biológicas, Postal Code 479, Pará State, Brazil

Rafael A Baraúna

Laboratório de Polimorfismo de DNA, Universidade Federal do Pará

Instituto de Ciências Biológicas, Postal Code 479, Pará State, Brazil

Maria PC Schneider

Laboratório de Polimorfismo de DNA, Universidade Federal do Pará

Instituto de Ciências Biológicas, Postal Code 479, Pará State, Brazil

Dr. Artur Silva

Laboratório de Polimorfismo de DNA, Universidade Federal do Pará

Instituto de Ciências Biológicas, Postal Code 479, Pará State, Brazil

Tel: 55-91-3201-8416 E-mail: asilva@ufpa.br

Received: September 12, 2012 Accepted: September 24, 2012 Published: January 1, 2013

doi:10.5296/ast.v1i1.2508 URL: http://dx.doi.org/10.5296/ast.v1i1.2508

Abstract

The Amazon region has several hydroelectric power reservoirs and, among these, is the Tucuruí reservoir, which has the fourth highest power in the world. This reservoir flooded a huge area of native forest, whose organic matter has been decomposed in the water column since then. This microbial process is carried out by several prokaryotic species, which present a great metabolic diversity, including among them methanotrophic bacteria and methanogens. In this study we sought to evaluate the community structure, composition and diversity along a water column of the Tucuruí reservoir located upstream of the dam by using denaturing high performance liquid chromatography (DHPLC) and 16S rDNA gene sequencing. Water samples were taken at intervals of 5 m from the surface down to a depth of 55 m along the water column. Our results show that the structures of communities of both *Archaea* and *Bacteria* remained constant down to a depth of 35 m indicating the existence of a stable environment until this depth. Changes in the abundance of some groups could be observed at the 40 m depth and the composition of the communities changed significantly at the depths of 45, 50 and 55 m. The deepest layer (55 m) presented the highest Archaeal diversity, while the highest Bacterial diversity was observed at the 0-40 m layers. *Crenarchaeota* were predominant in the upper layers, while *Euryarchaeota*, including methanogenic Archaea, were predominant in the 40 and 50 m layers, indicating the existence of an anoxic zone below 40 m. *Actinobacteria, Bacteroidetes* and *Proteobacteria* sequences were recovered from the superficial layers, as well as sequences not assigned to any phylum. Several sequences from the 55 m layer were assigned to *Dechloromonas*, known for the ability to degrade benzene and other aromatic compounds. We conclude that the structure and diversity of prokaryotic communities in the Tucuruí power reservoir is stable until the 35 m depth, changing in deeper layers.

Keywords: 16S rDNA, DHPLC, Amazon, Tucuruí dam, Bacterial Diversity

1. Introduction

Several environmental studies indicate hydroelectric power stations (HPS) as potential greenhouse gas emitters. Estimates indicate that these reservoirs emit about 321 Tg C. yr^{-1}, which account for 15-20% of the global warming potential of anthropogenic sources (St. Louis *et al*., 2000; Barros *et al*., 2011). Reservoirs located in the Amazon region have a higher potential of emitting greenhouse gases (Barros *et al*., 2011; Bastviken *et al*., 2011). However, the Amazonia is also the region of Brazil with the highest hydroelectric potential (52%) (Tundisi, 2007).

These hydroeletric powers reservoirs flood large forested areas, whose vegetation remains in decomposition under the water. The process of decomposition of organic matter is essentially a microbial one and involves the hydrolysis and fermentation or acidogenesis of complex polymers such as proteins, lipids and polysaccharides (Garcia *et al*., 2000). Among its bioproducts are simple substances, such as acetate, CO_2 and formate, which are used by methanogenic archaea as an energy source, with methane being produced in the last step of C mineralization (Gracia *et al*., 2000). Methane is produced in the sediment at the bottom of HPS reservoirs and remains dissolved in the water under high hydrostatic pressure, but it can reach the upper layers and be oxidized by methanotrophic bacteria (Ramos *et al*., 2009).

In environments rich in organic matter such as hydroelectric reservoirs, CO_2 is produced by syntrophic bacteria and methanotrophic bacteria as well (Conrad *et al*., 1999). On the other hand, methane is produced by methanogenic archaea through 3 pathways (acetoclastic, methylotrophic and hydrogenotrophic), but the contribution of the hydrogenotrophic pathway is lower in some aquatic environments (Conrad *et al*., 1999), which happens due to the presence of sulfate-reducing bacteria that compete by H_2:CO_2. The presence of methanogens of the orders *Methanosarcinales* and *Methanomicrobiales,* as well as of methanotrophs of the classes α- and γ-Proteobacteria, was reported recently in the anoxic zone of the Tucuruí HPS reservoir (Graças *et al*., 2011). In most bodies of water around the world, methane degradation is performed mainly by aerobic methanotrophic bacteria distributed from the oxic-anoxic interface to the surface (Blumenberg *et al*., 2007).

Cultivating many of these microbial groups is a laborious, unsuccessful task, which limits our knowledge about the microbial communities. Thus, culture-independent molecular techniques have been applied to provide information about these communities (Blumenberg *et al*., 2007; Barlaan *et al*., 2005; Wagner *et al*., 2009). Several studies use techniques such as denaturing gradient gel electrophoresis, temperature gradient gel electrophoresis, and single-strand conformation polymorphism, among others. Nevertheless, these techniques are labor-intensive and time demanding (Barlaan *et al*., 2005; Wagner *et al*., 2009). An alternative technique is the DHLPC, which is fast, highly reproducible, and has the advantage of decreasing the cost of randomly clone library sequencing (Barlaan *et al*., 2005, Wagner *et al*., 2009, Goldenberg *et al*., 2007; Xiao *et al*., 2001).

Despite the existence of several studies addressing greenhouse gas emissions by hydroelectric power reservoirs, few studies addressing microbes involved in the C cycle have been carried out in this type of environment (Graças *et al*., 2011; Dorador *et al*., 2007; Sekiguchi *et al*., 2002). Here we report changes in prokaryotic communities along a 55 m depth water column in the Tucuruí hydroelectric power reservoir, one of the main reservoirs in Brazil and the fourth largest in the world. Denaturing HPLC was used to generate community profiles and clone libraries were built to give us an insight into the taxonomic composition of our target communities, including those involved in the emission of greenhouse.

1.1 Research Methods

1.1.1 Site, Sampling and DNA Extraction

The Hydroelectric Power Station of Tucuruí is located in the state of Pará, Brazil. The area of its reservoir is of about 2,500 km^2 of flooded forest (which occurred in 1984). Water samples were taken in a single point (03°51'20,52"S/49°37'23,1"W), located 4 km away from the dam (Figure 1). A liter of water was sampled at intervals of 5 m with a van Dorn bottle from the surface to a depth of 55 m, comprising a total of 12 samples. A two-steps procedure was applied to filter the water. The water was passed through a qualitative paper (0.8 µm) in the first step, and then through a nitrocellulose membrane (0,22 µm) (Whatman, Germany) in the second step. The nitrocellulose membrane was cut into pieces and stored in DNA STE conservation buffer (50 mM Tris–HCl, 500 mM NaCl, 125 mM EDTA pH 8.0). The cells were centrifuged at 2,000 x g for 20 min, at 4ºC, and the supernatant was discarded. Total DNA was extracted with the UltraClean™ Soil DNA (MoBio, USA).

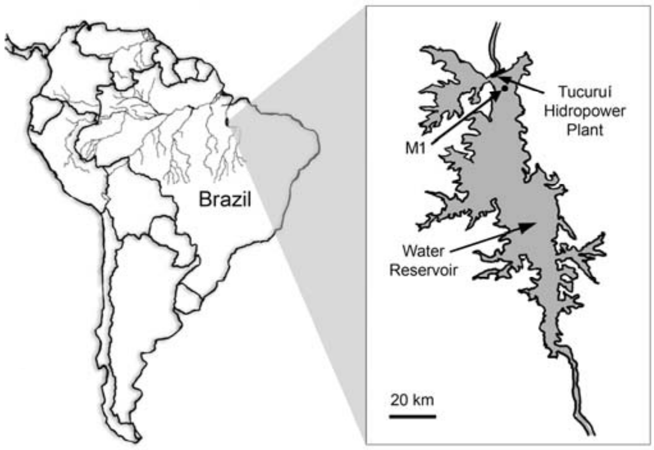


Figure 1. Geographic location of the Tucuruí hydroelectric power station reservoir

Description: The sampling point (M1) is displayed in the inset on the right side of the figure.

1.1.2 Polymerase Chain Reaction

The first PCR step was carried out with primers 16S-8F (5'- AGA GTT TGA T(CT)(AC) TGG CTC AG -3') and 16S-1407R (5'- GAC GGG GGT G(AT)G T(AG)C AA -3') to amplify the 16S rRNA bacterial gene, and primers 341F (5’- CCT A(CT)G GGG (CT)GC A(GC)C

AGG CG -3') and 1407R (5'- GAC GGG GGT G(AT)G T(AG)C AA -3') to the amplify the archaeal one. The thermal conditions for both PCRs were: an initial denaturation step of 95ºC for 5 min, 35 cycles of 95ºC for 1 min, 58ºC for 1 min and 72ºC for 1 min, and a final elongation step of 72ºC for 5 min.

The DHPLC only works with fragments smaller than 400 bp of length and the amplicons must have a GC clamp. Due to this limitation, another PCR was carried out using amplicons of the previous step as templates. The primers FP640 (5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG -3') and DHPLC 534R (5'-ATT ACC GCG GCT GCT GCT GG-3') were used to amplify bacterial genes, and the primers ARCHEAF GC 1100 (5'- CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CCG TCG ACA GTC AGG YAA CGA GCG AG -'3) and ARCHEAR 1400 (5'- CGG CGA ATT CGT GCA AGG AGC AGG GAC –'3) were used to amplify the archaeal genes. The thermal conditions for both PCRs were: an initial denaturation step of 95ºC for 5 min, 35 cycles of 95ºC for 1 min, 60ºC for 1 min and 72ºC for 1 min, and a final elongation step of 72ºC for 5 min. This second PCR generated amplicons of 200-300 bp of length corresponding to the V3 region of the 16S rRNA gene.

All PCRs had a volume of 50 µL the following composition: Buffer 1X, 2,5 mM $MgCl_2$, 0.2 mM dNTPs, 5 pM of each primer and 2 U Platinum Taq DNA polymerase – Invitrogen, USA.

1.1.3 DHPLC Analysis and Peak Selection

The separation of PCR products in the DHPLC is based on the partial elution of denatured DNA molecules and on the action of different gradients of the elution buffers passing through the column (Xiao *et al*., 2001). The phosphate groups of the DNA molecule interact with the polystyrene/divinylbenzene column, which is positively charged by triethylammonium (TEAA). The affinity of DNA by the column is decreased by the passage of acetonitrile under partial denaturation conditions. DNA with sequence variations have different retention times in the column. These differences are due to the low negative charge in single strand DNA if compared to double strand DNA (Barlaan *et al.,* 2005; Goldenberg *et al*., 2007).

The amplicons were run in the Transgenomic WAVE DHLPC system. Two systems of eluent buffers were used and fluorescence was used to detect the DNA fragments. Buffer A was composed of an aqueous solution of 0.1 M TEAA and pH 7.0, while buffer B was composed of 0.1 M TEAA and acetonitrile 25% (v:v). Sequences were separated according to their retention time in the polystyrene/divinylbenzene column. The following parameters were used for Bacteria: column temperature of 65ºC, with an initial rate of buffer B of 35%, reaching a rate of 72% after 17 min. The parameters used for Archaea were the same as those used for Bacteria, except for the column temperature, which was of 63 ºC.

Non redundant peaks were selected and their amplicons collected for the construction of clone libraries using the TA cloning kit (Invitrogen, USA) and sequencing in the ABI 3730 xl (Applied Biosystems, USA) platform according to the manufacturer's manual. This approach was used because we assumed that each peak contain several bacterial and archaeal species. Two libraries were built, one for Archaea with 202 sequences, containing an average of 17 clones per peak, and another for Bacteria with 149 sequences, containing an average of 12 clones per peak. The sequences were deposited in the EMBL database under accession numbers HE602025-HE602369 and HE613183-HE613188.

1.1.4 Data Analysis

Partial sequences of the 16S rRNA gene were edited with Bioedit. Potential chimeras were identified with the Bellerophon function of the program Mothur (Schloss *et al.*, 2009) and discarded. The taxonomical classification of the sequences was performed with the Classifier RDP tool (Cole *et al.*, 2009), considering a threshold of 80%. Sequences with boostrap below this threshold were considered as unclassified. The sequences were aligned based on their secondary structure with Mothur and used to calculate a distance matrix (Jukes-Cantor method). Operational taxonomic units (OTUs) were defined at a distance of 0.03 and representative sequences were selected for each OTU. These representative sequences were used for phylogenetic analysis with sequences recovered from the RDP database by using the Seqmatch tool. Neighbor-joining trees were built with MEGA v.5.0 (Tamura *et al.*, 2011), by using the Jukes-Cantor distance and 1000 bootstrap replicates. In order to simplify the tree presentation only one OTU was maintained in a clade when more than one representative OTU was in that clade.

2. Results

2.1 Community Structure as Evaluated by DHPLC

The DHPLC chromatograms of bacterial 16S rRNA genes present peaks with similar retention times and heights in a depth range of 0 to 35 m, indicating that the structures of the bacterial communities remained constant down to a depth of 35 m (Figure 2B). The abundance of amplicons represented by each one of the peaks did not differ among these chromatograms, as indicated by a chi-square analysis ($P>0.05$). The community starts to change at the 40 m depth, where the 9.5 min retention time peak (D1) disappears. Richness is extremely reduced at the 45 m depth layer, as indicated by the presence of a single peak in its chromatogram (G1). A drastic change in community structure is observed again at the 50 m depth layer, with a significant reduction in the height of the 7.5 min peak (G1) and the appearance of new peaks, including two peaks with retention times of 9.6 and 9.8 min (D1 and E1), which are also observed at the 55 m depth layer.

Similarly to what was observed to *Bacteria*, the chromatograms of archaeal 16S rRNA genes remained the same in a depth range of 0 to 35 m (Figure 2B). The archaeal community also started to change at the 40 m depth, as indicated by changes in the abundance of the peaks. The community changes drastically at the 45 m depth. The chromatograms at the 50 and 55 m depths are completely different from the others. Additionally, the number of peaks is significantly increased at 55 m, indicating that archaeal richness is the highest at this depth.

The chromatograms show that archaeal communities have the same structure from the surface down to the depth of 35 m. These chromatograms present only three peaks, indicating 3 dominant groups of amplicons (Figure 2A). Community structure at 40 m is somewhat similar to that of the upper layers, but a shift in the abundance of the dominant members can be observed as indicated by shifts in the height of peaks. A pronounced change in community structure can be observed at 45 m, and significant changes occur at each of the underlying layers (50 and 55 m), as indicated by their completely different chromatograms. Five peaks were observed at 55 m, showing the presence of 5 dominant groups.

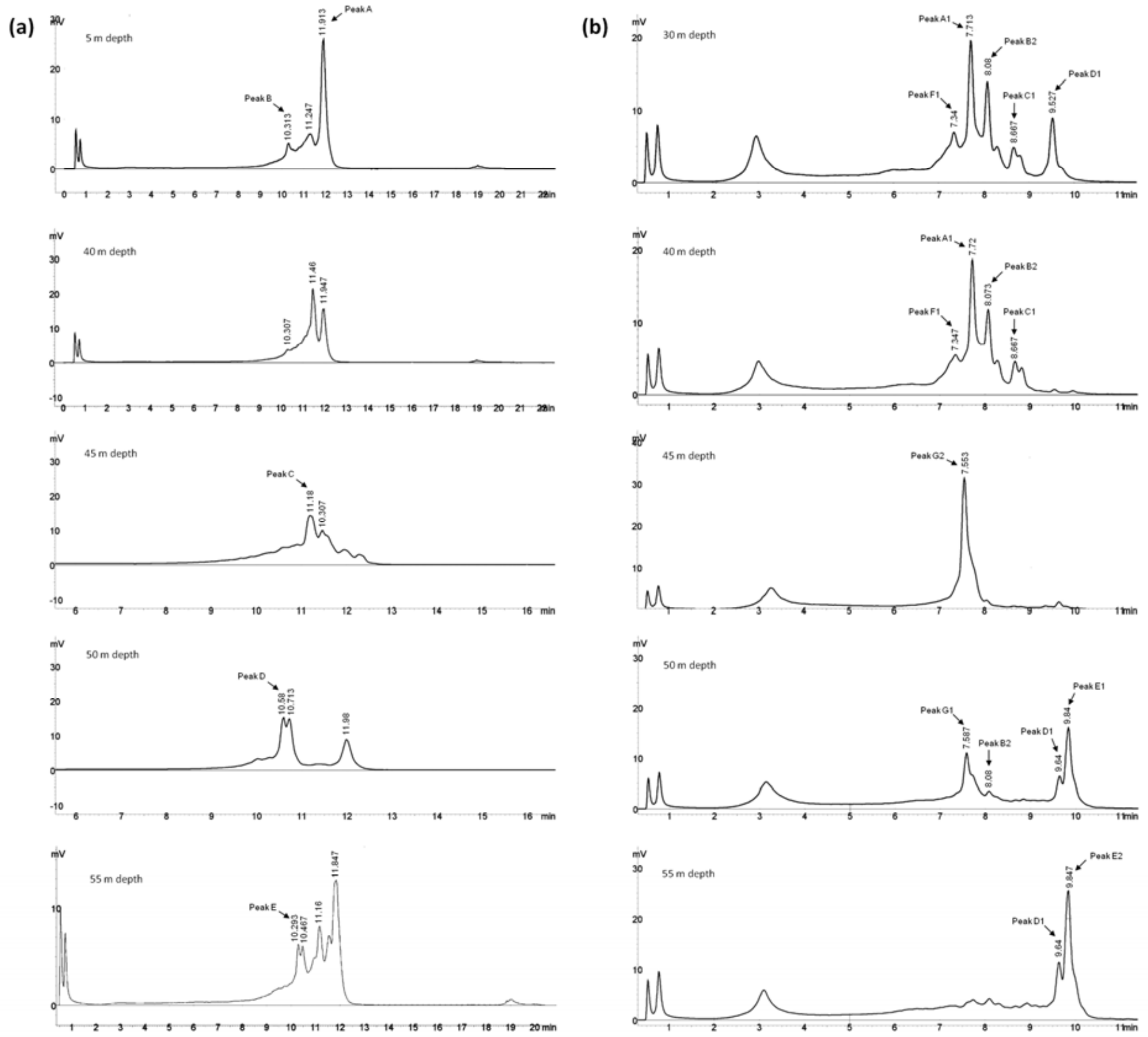


Figure 2. DHPLC chromatograms of archaeal (A) and bacterial (B) communities in a water column of the Tucuruí hydroelectric power station reservoir

Description: Only one chromatogram is displayed to represent communities at the depths of 0 to 35 m since chromatrograms of these communities are the same. The letters indicate peaks whose amplicons were sampled for cloning and sequencing.

2.2 Phylogenetic Analysis

Some peaks were selected and their amplification products were cloned and sequenced. About 20 clones per peak were picked randomly for sequencing. These sequences were used for taxonomic assignment with the RDP Classifier tool and included into phylogenies to better resolve their phylogenetic relationships.

Five nonredundant peaks from the archaeal communities were sequenced. These peaks are indicated with letters from A to E in Figure 2A. Twenty-three percent of these sequences were classified as belonging to *Crenarchaeota* and 53% as belonging to *Euryarchaeota*. Twenty-four percent of the sequences were not classified into any phyla. *Crenarchaeota* were predominant in the upper layers, while *Euryarchaeota*, known for containing methanogenic species, were predominant in the 40 and 50 m layers (Figure 3). Seventy percent of the *Euryarchaeota* were classified as *Methanomicrobiales* (Figure 3).

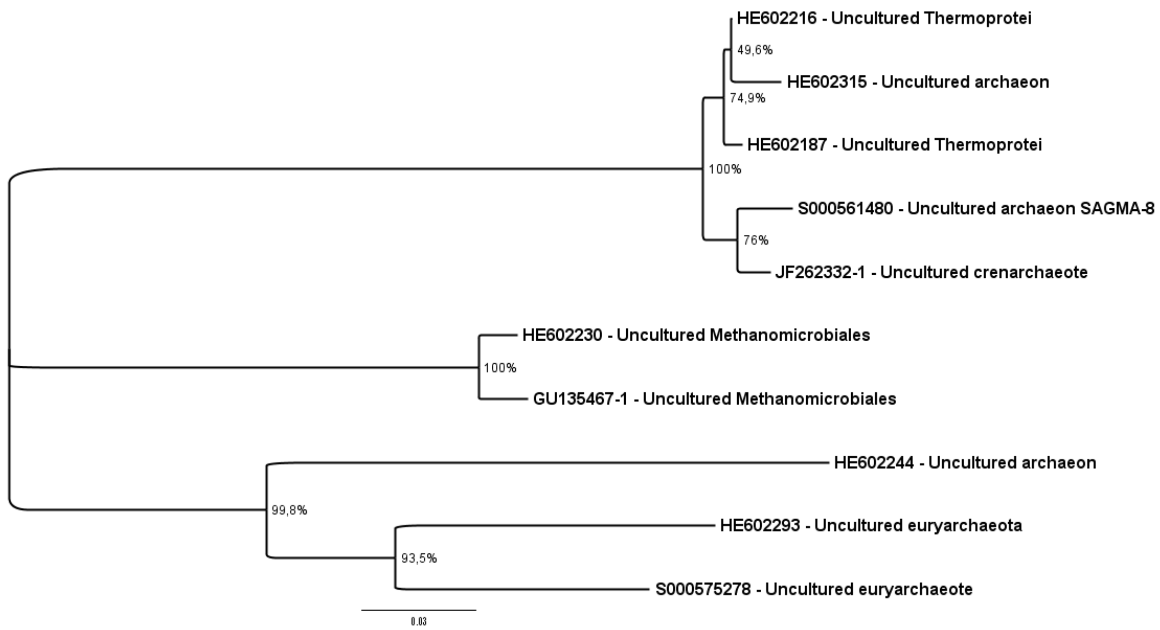


Figure 3. Unrooted neighbor-joining phylogenetic tree of 16S rRNA archaeal sequences

Description: The three clades displayed on the tree are *Crenarchaeota*, *Euryarchaeota* and methanogens of the order *Methanomicrobiales*. The tree was calculated with MEGA, with 1,000 bootstrap replicates using the Jukes-Cantor model.

Seven nonredundant peaks were sequenced for Bacteria (A1, B2, C1, D1, E1, F1, G1). The clones were classified as *Proteobacteria* (34%), *Actinobacteria* (11%) and *Bacteroidetes* (6%). Forty-six percent of the sequences remained unclassified. *Nitrospira*, SR1 and *Firmicutes* comprised 1% of the sequences each (Figure 4). Histograms displaying the diversity of each peak are presented in the supplemental material.

Aquatic Science and Technology
ISSN 2168-9148
2013, Vol. 1, No. 1

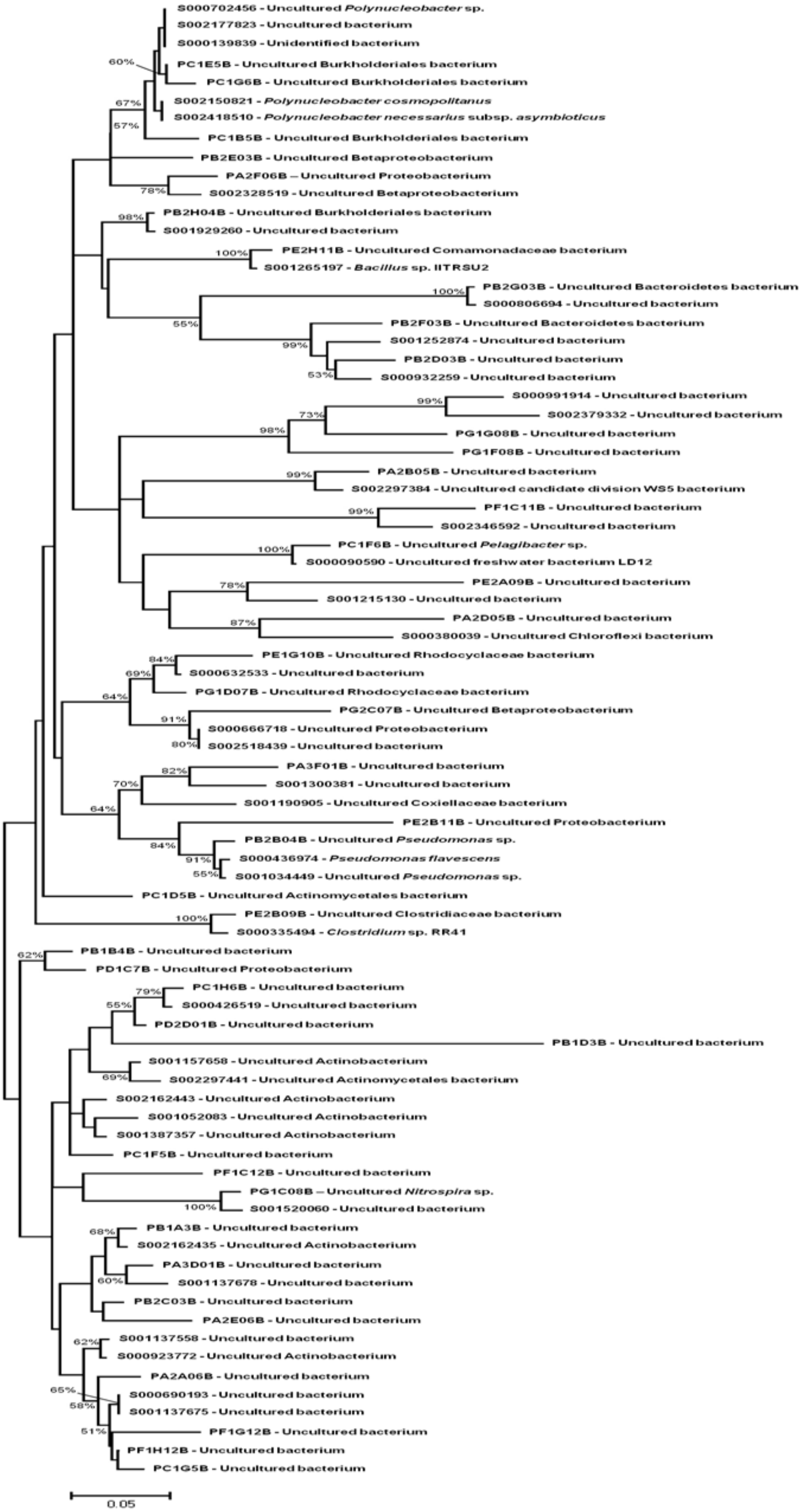


Figure 4. Unrooted neighbor-joining phylogenetic tree of 16S rRNA bacterial sequences

Description: The tree was calculated with MEGA, with 1,000 bootstrap replicates using the Jukes-Cantor model.

Communities were very similar from 0-40 m, presenting a large number of unclassified bacteria, followed by bacteria belonging to *Actinobacteria* and *Proteobacteria* (essentially α, β and γ-*Proteobacteria*). Different phyla were found at 45 m. *Nitrospira* was found at 45 m and β-*Proteobacteria* (family *Rhodocyclaceae*) predominated at this layer. These *Rhodocyclaceae* sequences presented a hit with low support for the genus *Dechloromonas*. β-*Proteobacteria* and γ-*Proteobacteria* also predominated at 50 and 55 m, comprising up to 90% of the sequences found at the 55 m layer. *Firmicutes* were found only in these deeper layers. Several sequences from the 55 m layer were assigned to *Dechloromonas*.

3. Discussion

Even though we did not measure physicochemical variables at the different depths, the similarity of communities in the 0-35 m depth range indicates that the environmental conditions until 35 m are similar to those found at the upper layers and in the surface, which involves high oxygen concentration and nutrients, indicating a mixture of the water in these layers. Both bacterial and archaeal data present the same pattern of change, which gives more support to this conclusion. Changes in the structure of the microbial communities at the depth of 40 m indicate that this is a layer of transition to a possibly anoxic zone represented by the layers of 45, 50 and 55 m depth. This conclusion is supported by the shift in community composition from *Actinobacteria* and *Proteobacteria*, which contain several aerobic species, in the layers from 0 to 35 m depth, to the presence of methanogenic *Archaea*, which are strictly anaerobic, in the deepest layers of 45 to 55 m depth.

The predominance of *Crenarchaeota* in the upper layers was previously reported (Graças *et al.*, 2011). All *Crenarchaeota* were highly similar to the groups MG1 or SAGMA-8, which are classified as *Thermoprotei* and frequently found in the ocean floor and in mines of South Africa, but they can also be found in non-extreme environments such as lakes (Graças *et al.*, 2011; MacGregor *et al.*, 1997; Schleper *et al.*, 1997; Schleper *et al.*, 2005), although at a lower frequency. This diversity of habitats illustrates the diversity of this phylum, although its metabolism remains unclear (Schleper *et al.*, 2005). *Crenarchaeota* were found in all peaks, expect for peak D, and the majority (~70%) were found in peak A (supplemental).

The unclassified *Euryarchaeota* were similar to those found in soils cultivated with rice and wheat, and in marine environmental samples. Some sequences were assigned by the RDP Classifier to methanogenic *Archaea* of the orders *Methanomicrobiales* and *Thermoplasmatales* with a high bootstrap support (80-97%). However, sequences restricted to peak C, found only at the depth of 50 m, had a bootstrap support lower than 80%, indicating the presence of distinct groups at this depth. A higher and yet little characterized diversity of *Euryarchaeota* is expected at deepest layers such as this due to the richness of substrate in those layers. Dumestre *et al.* (2001) reported *Archaea* belonging to the same orders in the reservoir of the UHE Petit Saut.

The commonest and best-characterized class of *Euryarchaeota* in our analysis was *Methanomicrobiales*, which was already reported in the same reservoir in the deep aphotic (40 m) layer and sediment (Graças *et al.*, 2011). Methanogenic *Archaea* are frequently divided into 5 classes (*Methanomicrobiales*, *Methanococcales*, *Methanosarcinales*, *Methanobacteriales* e *Methanopyrales*), based on 16S rRNA phylogenies, but there are results indicating that they should be divided into three classes, and class II is represented by *Methanomicrobiales* (Anderson *et al.*, 2009). This order uses the hydrogenotrophic pathway to produce methane, and prefers low environmental H_2 concentrations. Methanogenic *Archaea* of the order *Methanomicrobiales* are phylogenetically closer to *Methanosarcinales*, but share several genes with Class I, making them metabolically closer to Class I. Indeed, *Methanosarcinales* predominates in environments where acetate is abundant (Anderson *et al.*, 2009).

Unclassified *Archaea* were found in all peaks, but those were more abundant in peak D. This shows that a great part of the archaeal diversity is not known in the reservoir and that it is a potential source of unknown species. The presence of unclassified *Archaea* in Amazon environments was reported several times in soils (Borneman & Triplet, 1997; Navarrete *et al.*, 2010) and in sediments (Araújo, 2010; Conrad *et al.*, 2010), but a few studies report this in freshwaters or rivers (Graças *et al*., 2011).

The least abundant phyla were SR1, *Firmicutes* and *Nitrospira*. Reads belonging to these phyla accounted for only 3% of the total reads. *Nitrospira* and *Firmicutes* were not found in the layers above 45 m. These phyla were also found by Graças *et al*. (2011) in a zone considered as anoxic in the same reservoir.

Representative OTUs of *Firmicutes* were assigned to *Clostridia*. *Nitrospira* was found only in peak G1. All sequences belonging to this phylum were highly similar and were grouped into a single OTU. *Nitrospira* is involved in the nitrogen cycle and are amongst the most abundant and diverse chemolithoautotrophic nitrite-oxidizing bacteria (NOB). Most of these bacteria are unculturable and can be reported in several environments (Altman *et al.*, 2003; Freitag *et al.*, 2005). Rencently, the first genome of a strain of this phylum, Candidatus *Nitrospira defluvii*, was sequenced from a metagenomic library enriched with this bacterium [27].

Reads of the candidate division SR1 were detected until the depth of 40 m. The fraction of this division in microbial communities varies among different environments (Davis *et al.,* 2009). Nevertheless, they usually represent a small fraction of these communities. They present a high phylogenetic diversity, but a limited morphological diversity (Davis *et al.*, 2009). They are not restricted to fresh water, but can be also found in marine sediment (van der Wielen *et al.*, 2005) and in the gut of animals (Ley *et al.*, 2008). Borrel *et al.* (2010) also observed a distribution of these bacteria along a water column in Lake Pavin, France, which also presents an anoxic zone.

Eleven percent of the sequences were assigned to *Actinobacteria* and a large part of these sequences were assigned to the order *Actinomycetales*. This phylum presents a great biochemical and morphological diversity, as well as different forms of respiration (Embley *et al.*, 1994; Hayakawa *et al.*, 2000). These bacteria are distributed in terrestrial (Hayakawa *et al.*, 2000; Heuer *et al.*, 1997; McVeigh *et al.*, 1996), fresh water (Newton *et al.,* 2006; Schauer *et al.*, 2006; Wu *et al.*, 2007) and marine (Clementino *et al.*, 2008; Demergasso *et al.*, 2004; Oren, 2008) environments.

Close to 6% of the total diversity of bacteria was represented by *Bacteroidetes*, a phylum containing mainly strict anaerobic bacteria usually associated to the gut. A large amount of these sequences were assigned to the genus *Provetella*, whose presence was previously related to impacted environments (Okabe *et al.*, 2007). Others had high identity to the order *Sphingobacteria*, but with few hits. Members of this order are important to the mineralization of complex polymers, and are frequently found where there is deposition of organic matter. They can be found either in fresh water and marine environments, as well as in sediments and soils (Newton *et al.*, 2006; Schauer *et al.*, 2006; Wu *et al.*, 2006; Lydell *et al.*, 2004). *Bacteroidetes* presented the fastest response to the addition of complex organic matter in sediments under strict anaerobic conditions, suggesting a role in the provision of organic composts of low molecular weight that would be mineralized by sulphate-reducing bacteria (Rosello-Mora *et al.*, 1999).

Proteobacteria was the most abundant phylum (64% of the sequences). These bacteria are commonly found in water and sediments of saline, alkaline and freshwater lagoons (Glocker *et al.*, 2000; Hirons *et al.*, 1997; Humayoun *et al.*, 2003; Koizumi *et al.*, 2004). The abundance of Proteobacterial sequences with depth and of *Archaea* as well, can be related to low iron and sulphate concentrations in anoxic zones, limiting the availability of electron acceptors (Dong *et al.*, 2006). These scarcity forces the selection of microorganisms with alternative pathways such as methanogenesis and methanotrophy, present in *Archaea* and *Protebacteria* (α-*Proteobacteria* e γ-*Proteobacteria*), respectively.

The predominance of *Proteobateria* (essentially orders *Burkholderiales* and *Rhodocyclales*) in deeper layers may also be due to the increase in the concentration of organic matter (Lamontagne *et al.*, 2004; Labbé *et al.*, 2007), which increases the production and consumption rates of methane and organic matter degradation by genera belonging to α-*Proteobacteria* and that use a great diversity of C1 substances (Layton *et al.*, 2000; Rainey *et al.,* 1998). β-*Proteobacteria*, especially those belonging to orders *Burkholderiales* and *Rhodocyclales* were predominant at different depths. A variety of bacteria belonging to these orders are able to degrade chlorinated and aromatic compounds (Kasai *et al.*, 2005; Hamamura *et al.*, 2006; Yuste *et al.*, 2000).

Other classes found were γ-*Proteobacteria* (mainly *Pseudomonadales*) and α-*Proteobacteria* (essentially *Rickettsiales*), which include bacteria involved in methane utilization for maintaining their metabolism, raising an important environment potential (Rees *et al.*, 2004; Teske *et al.*, 2002).

4. Conclusions

The structure of prokaryotic communities remained unchanged from the 0 m to the depth of 35 m. Bacterial communities became less diverse and archaeal communities more diverse at layers below 35 m. *Proteobacteria*, *Actinobacteria*, *Euryarchaeota*, *Crenarchaeota* and uncultured prokaryotes were predominant. The presence and abundance of methanogens in the deeper layers indicates the presence of an anoxic zone, with the consequent production of methane, at the depth of 40 m and below.

Acknowledgement

This study is part of the Research and Development Programme of the Electrical Energy Sector regulated by ANEEL and Centrais Elétricas do Norte do Brasil. The authors are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (National Council for Scientific and Technological Development–Brazil).

References

Altman, D., Stief, P., Amann, R., Beer, D., & Schramm, A. (2003). *In situ* distribution and activity of nitrifying bacteria in freshwater sediment. *Environmental Microbiology*, *5*(9), 798-803. http://dx.doi.org/10.1046/j.1469-2920.2003.00469.x

Anderson, I., Ulrich, L. E., Lupa, B., Susanti, D., Porat, I., et al. (2009). Genomic characterization of methanomicrobiales reveals three classes of methanogens. *PloS one, 4*, e5797. http://dx.doi.org/10.1371/journal.pone.0005797

Araújo, A. C. V. (2010). Diversidade molecular de arquéias em sedimentos de rios da amazônia e caracterização de espécies metanogênicas cultivadas. Universidade de São Paulo. Thesis, 123p.

Barlaan, E. A., Sugimori, M., Furukawa, S., & Takeuchi, K. (2005). Profiling and monitoring of microbial populations by denaturing high-performance liquid chromatography. *Journal of microbiological methods, 61*, 399-412. http://dx.doi.org/10.1016/j.mimet.2005.01.002

Barros, N., Cole, J. J., Tranvik, L. J., Prairie, Y. T., Bastviken, D., et al. (2011). Carbon emission from hydroelectric reservoirs linked to reservoir age and latitude. *Nature Geoscience, 4*, 1-4. http://dx.doi.org/10.1038/ngeo1211

Bastviken, D., Tranvik, L. J., Downing, J. A., Crill, P. M., & Enrich-Prast, A. (2011). Freshwater Methane Emission Offset the Continental Carbon Sink. *Science, 331*, 50. http://dx.doi.org/10.1126/science.1196808

Blumenberg, M., Seifert, R., & Michaelis, W. (2007). Aerobic methanotrophy in the oxic–anoxic transition zone of the Black Sea water column. *Organic Geochemistry, 38*, 84-91. http://dx.doi.org/10.1016/j.orggeochem.2006.08.011

Borneman, J., & Triplett, E. W. (1997). Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Applied and environmental microbiology, 63*, 2647-2653.

Borrel, G., Lehours, A. C., Bardot, C., Bailly, X., & Fonty, G. (2010). Members of candidate divisions OP11, OD1 and SR1 are widespread along the water column of the meromictic Lake Pavin (France). *Arch Microbiol, 192*, 559-567. http://dx.doi.org/10.1007/s00203-010-0578-4

Clementino, M. M. et al. (2008). Prokaryotic diversity in one of the largest hypersaline coastal lagoons in the world. *Extremophiles, 12*, 595-604. http://dx.doi.org/10.1007/s00792-008-0162-x

Cole, J. R., Wang, Q., Cardenas, E., Fish, J., Chai, B., et al. (2009). The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic acids research, 37*, D141-D145. http://dx.doi.org/10.1093/nar/gkn879

Conrad, R. (1999). Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiology Ecology*, *28*, 193-202. http://dx.doi.org/10.1111/j.1574-6941.1999.tb00575.x

Conrad, R., Klose, M., Claus, P., & Enrich-Prast, A. (2010). Methanogenic pathway, 13 C isotope fractionation, and archaeal community composition in the sediment of two clear-water lakes of Amazonia. *Limmol Oceanogr*, *55*, 689-702. http://dx.doi.org/10.4319/lo.2009.55.2.0689

Davis, J. P., Youssef, N. H., & Elshahed, M. S. (2009). Assessment of the diversity, abundance, and ecological distribution of members of candidate division SR1 reveals a high level of phylogenetic diversity but limited morphotypic diversity. *Applied and Environmental Microbiology, 75*(12), 4139-4148. http://dx.doi.org/10.1128/AEM.00137-09

Demergasso, C., et al. (2004). Distribution of prokaryotic genetic diversity in athalassohaline lakes of the Atacama Desert, Northern Chile. *FEMS Microbiology Ecology*, *48*, 57-69. http://dx.doi.org/10.1016/j.femsec.2003.12.013

Dong, H., Zhang, G., Jiang, H., Yu, B., Chapman, L. R., et al. (2006). Microbial Diversity in Sediments of Saline Qinghai Lake, China: Linking Geochemical Controls to Microbial Ecology. *Microbial Ecology*, *51*, 65-82. http://dx.doi.org/10.1007/s00248-005-0228-6

Dorador, C., Castillo, G., Witzel, K., & Vila, I. (2007). Bacterial diversity in the sediments of a temperate artificial lake, Rapel reservoir. *Revista Chilena de Historia Natural*, 213-224.

Dumestre, J. F., Casamayor, E. O., Massana, R., & Pedrós-alió, C. (2001). Changes in bacterial and archaeal assemblages in an equatorial river induced by the water eutrophication of Petit Saut dam reservoir (French Guiana). *Aquatic Microbial Ecology*, *26*, 209-221. http://dx.doi.org/10.3354/ame026209

Embley, T. M. & Stackebrandt, E. (1994). The molecular phylogeny and systematics of the actinomycetes. *Annual review of microbiology*, *48*, 257-289. http://dx.doi.org/10.1146/annurev.micro.48.1.257

Freitag, T. E., Chang, L., Clegg, C. D., & Prosser, J. I. (2005). Influence of Inorganic Nitrogen Management Regime on the Diversity of Nitrite-Oxidizing Bacteria in Agricultural Grassland Soils Influence of Inorganic Nitrogen Management Regime on the Diversity of Nitrite-Oxidizing Bacteria in Agricultural Grassland Soil. *Applied and Environmental Microbiology*, *71*(12), 8323-8334. http://dx.doi.org/10.1128/AEM.71.12.8323-8334.2005

Garcia, J. L., Patel, B. K. C., & Ollivier, B. (2000). Taxonomic phylogenetic and ecological diversity of methanogenic Archaea. *Anaerobe*, *6*, 205-226. http://dx.doi.org/10.1006/anae.2000.0345

Glockner, F. O., Zaichikov, E., Belkova, N., Denissova, L., Pernthaler, J., et al. (2000).

Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. *Appl Environ Microbiol*, *66*, 5053-5065. http://dx.doi.org/10.1128/AEM.66.11.5053-5065.2000

Goldenberg, O., Herrmann, S., Marjoram, G., Noyer-Weidner, M., Hong, G., et al. (2007). Molecular monitoring of the intestinal flora by denaturing high performance liquid chromatography. *Journal of microbiological methods*, *68*, 94-105. http://dx.doi.org/10.1016/j.mimet.2006.06.009

Graças, D. A., Miranda, P. R., Baraúna, R. A., McCulloch, J. A., Ghilardi, R., et al. (2011). Microbial Diversity of an Anoxic Zone of a Hydroelectric Power Station Reservoir in Brazilian Amazonia. *Microb Ecol*, *62*, 853-861. http://dx.doi.org/10.1007/s00248-011-9906-8

Hamamura, N., Olson, S. H., Ward, D. M., & Inskeep, W. P. (2006). Microbial population dynamics associated with crude-oil biodegradation in diverse soils. *Appl Environ Microbiol*, *72*, 6316-6324. http://dx.doi.org/10.1128/AEM.01015-06

Hayakawa, M., Otoguro, M., Takeuchi, T., Yamazaki, T., & Limura, Y. (2000). Application of a method incorporating differential centrifugation for selective isolation of motile actinomycetes in soil and plant litter. *Antonie van Leeuwenhoek*, *78*, 171-185. http://dx.doi.org/10.1023/A:1026579426265

Heuer, H., Krsek, M., Baker, P., Smalla, K., & Wellington, E. M. H. (1997). Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Appl Environ Microbiol*, *63*, 3233-3241.

Hirons, W. D., Methe, B. A., Nierzwicki-Bauer, S. A., & Zehr, J. P. (1997). Bacterial diversity in Adirondack Mountain lakes as revealed by 16S rRNA gene sequences. *Appl Environ Microbiol*, *63*, 2957-2960.

Humayoun, S. B., Bano, N., & Hollibaugh, J. T. (2003). Depth distribution of microbial diversity in Mono Lake, a meromictic soda lake in California. *Appl Environ Microbiol*, *69*, 1030-1042. http://dx.doi.org/10.1128/AEM.69.2.1030-1042.2003

Kasai, Y., Takahata, Y., Hoaki, T., & Watanabe, K. (2005). Physiological and molecular characterization of a microbial community established in unsaturated, petroleum-contaminated soil. *Environ Microbiol*, *7*, 806–818. http://dx.doi.org/10.1111/j.1462-2920.2005.00754.x

Koizumi, Y., Kojima, H., & Fukui, M. (2004). Dominant microbial composition and its vertical distribution in saline meromictic Lake Kaiike (Japan) as revealed by quantitative oligonucleotide probe membrane hybridization. *Appl Environ Microbiol*, *70*, 4930-4940. http://dx.doi.org/10.1128/AEM.70.8.4930-4940.2004

Lamontagne, M. G., Leifer, I., Bergmann, S., Vandewerfhorst, L. C., & Holden, P. A. (2004). Bacterial diversity in marine hydrocarbon seep sediment. *Environ Microbiol*, *6*, 799-808. http://dx.doi.org/10.1111/j.1462-2920.2004.00613.x

Labbé, D., Margesin, R., Schinner, F., Whyte, L. G., & Greer, C. W. (2007). Comparative phylogenetic analysis of microbial communities in pristine and hydrocarbon contaminated Alpine soils. *FEMS Microbiol Ecol*, *59*, 466-475. http://dx.doi.org/10.1111/j.1574-6941.2006.00250.x

Layton, A. C., et al. (2000). Quantification of Hyphomicrobium populations in activated sludge from an industrial wastewater treatment system as determined by 16S rRNA analysis. *Appl Environ Microbiol*, *66*, 1167-1174. http://dx.doi.org/10.1128/AEM.66.3.1167-1174.2000

Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R., & Gordon, J. I. (2008). Worlds within worlds: evolution of the vertebrate gut microbiota. *Nature Reviews Microbiology*, *6*(10), 776-88. http://dx.doi.org/10.1038/nrmicro1978

Lücker, S., et al. (2010). A Nitrospira metagenome illuminates the physiology and evolution of globally important nitrite-oxidizing bacteria. *PNAS*, *107*(30), 13479-84. http://dx.doi.org/10.1073/pnas.1003860107

Lydell, C., Dowell, L., Sikaroodi, M., Gillevet, P., & Emerson, D. (2004). A Population Survey of Members of the Phylum Bacteroidetes Isolated from Salt Marsh Sediments along the East Coast of the United States. *Microbial Ecology, 48*(2), 263-273. http://dx.doi.org/10.1007/s00248-003-1068-x

MacGregor, B. J., Moser, D. P., Alm, E. W., Nealson, K. H., & Stahl, D. A. (1997). Crenarchaeota in Lake Michigan Sediment. *Applied and Environmental Microbiolgy*, *63*, 1178-1181.

McVeigh, H. P., Munro, J., & Embley, T. M. (1996). Molecular evidence for the presence of novel actinomycete lineages in a temperate forest soil. *J Ind Microbiol, 17*, 197-204. http://dx.doi.org/10.1007/BF01574693

Navarrete, A. A., Cannavan, F. S., Taketani, R. G., & Tsai, S. M. (2010). A Molecular Survey of the Diversity of Microbial Communities in Different Amazonian Agricultural Model Systems. *Diversity*, *2*, 787-809. http://dx.doi.org/10.3390/d2050787

Newton, R. J., Kent, A. G., Triplett, E. W., & McMahon, K. D. (2006). Microbial community dynamics in a humic lake: differential persistence of common freshwater phylotypes. *Environmental Microbiology*, *8*(6), 956-970. http://dx.doi.org/10.1111/j.1462-2920.2005.00979.x

Okabe, S., Okayama, N., Savichtcheva, O., & Ito, T. (2007). Quantification of host-specific Bacteroides–Prevotella 16S rRNA genetic markers for assessment of fecal pollution in freshwater. *Appl Microbiol Biotechnol*, *74*, 890-901. http://dx.doi.org/10.1007/s00253-006-0714-x

Oren, A. (2008). Microbial life at high salt concentrations: phylogenetic and metabolic diversity. *Saline Systems*, *4*(2).

Rainey, F. A., Ward-Rainey, N., Gliesche, C. G., & Stackebrandt, E. (1998). Phylogenetic analysis and intrageneric structure of the genus Hyphomicrobium and the related genus

Filomicrobium. *Int J Syst Bacteriol*, *48*, 635-639. http://dx.doi.org/10.1099/00207713-48-3-635

Ramos, F. M., et al. (2009). Methane stocks in tropical hydropower reservoirs as a potential energy source. *Climatic Change*, *93*, 1-13. http://dx.doi.org/10.1007/s10584-008-9542-6

Rees, H. C., Grant, W. D., Jones, B. E., & Heaphy, S. (2004). Diversity of Kenyan soda lake alkaliphiles assessed by molecular methods. *Extremephiles*, *8*, 63-71. http://dx.doi.org/10.1007/s00792-003-0361-4

Rosello-Mora, R., Thamdrup, B., Schafer, H., Weller, R., & Amann, R. (1999). The response of the microbial community of marine sediments to organic carbon input under anaerobic conditions. *Syst Appl Microbiol*, *22*, 237-248. http://dx.doi.org/10.1016/S0723-2020(99)80071-X

Schauer, M., Jiang, J., & Hahn, M. (2006). Recurrent Seasonal Variations in Abundance and Composition of Filamentous SOL Cluster Bacteria (*Saprospiraceae*, *Bacteroidetes*) in Oligomesotrophic Lake Mondsee (Austria). *Applied and Environmental Microbiology, 72*(7), 4704-4712. http://dx.doi.org/10.1128/AEM.02935-05

Schleper, C., Holben, W., & Klenk, H. P. (1997). Recovery of Crenarchaeotal Ribosomal DNA Sequences from Freshwater-Lake Sediments. *Applied and Environmental Microbiolgy*, *63*, 321-323.

Schleper, C., Jurgens, G., & Jonuscheit, M. (2005). Genomic Studies of Uncultivated Archaea. *Nature Reviews Microbiology*, *3*, 479-488. http://dx.doi.org/10.1038/nrmicro1159

Schloss, P. D., et al. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and environmental microbiology*, *75*, 7537-7541. http://dx.doi.org/10.1128/AEM.01541-09

Sekiguchi, H., et al. (2002). Bacterial distribution and phylogenetic diversity in the Changjiang estuary before the construction of the Three Gorges Dam. *Microbial ecology*, *43*, 82-91. http://dx.doi.org/10.1007/s00248-001-0034-8

St. Louis, V., Kelly, C. A., Duchemin, E., Rudd, J. W., & Rosenberg, D. M. (2000). Reservoir Surfaces as Sources of Greenhouse Gases to the Atmosphere : A Global Estimate. *BioScience*, *50*, 766-775. http://dx.doi.org/10.1641/0006-3568(2000)050[0766:RSASOG]2.0.CO;2

Stackebrandt, E., Rainey, F. A., & Ward-Rainey, N. L. (1997). Proposal for a New Hierarchic Classification System, Actinobacteria classis nov. *International journal of systematic bacteriology*, *47*, 479-491. http://dx.doi.org/10.1099/00207713-47-2-479

Tamura, K., et al. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular biology and evolution*, *28*, 2731-2739. http://dx.doi.org/10.1093/molbev/msr121

Teske, A., et al. (2002). Microbial diversity of hydrothermal sediments in the Guaymas Basin:

evidence for anaerobic methanotrophic communities. *Appl. Environ. Microbiol*, *68*, 1994-2007. http://dx.doi.org/10.1128/AEM.68.4.1994-2007.2002

Tundisi, J. G. (2007). Exploraçao do potencial hidrelétrico da Amazônia. *Estudos Avançados*, *21*, 109-117. http://dx.doi.org/10.1590/S0103-40142007000100009

van der Wielen, P. W. J. J., *et al*. (2005). The enigma of prokaryotic life in deep hypersaline anoxic basins. *Science*, *307*(5706), 121-3. http://dx.doi.org/10.1126/science.1103569

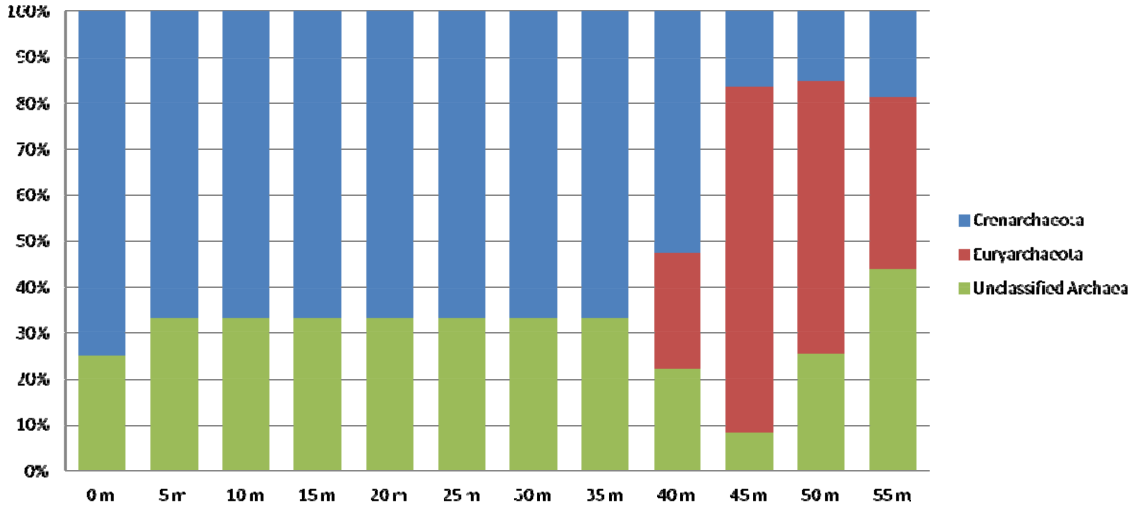
Wagner, A. O., Malin, C., & Illmer, P. (2009). Application of denaturing high-performance liquid chromatography in microbial ecology: fermentor sludge, compost, and soil community profiling. *Applied and Environmental Microbiology*, *75*, 956-964. http://dx.doi.org/10.1128/AEM.01411-08

Wu, X., Xi, W., Ye, W., & Yang, H. (2007). Bacterial community composition of a shallow hypertrophic freshwater lake in China, revealed by16S rRNA gene sequences. *FEMS Microbiol Ecol*, *61*, 85-96. http://dx.doi.org/10.1111/j.1574-6941.2007.00326.x

Xiao, W., & Oefner, P. J. (2001). Denaturing High-Performance Liquid Chromatography: A Review. *Human Mutation*, *17*, 439-474. http://dx.doi.org/10.1002/humu.1130

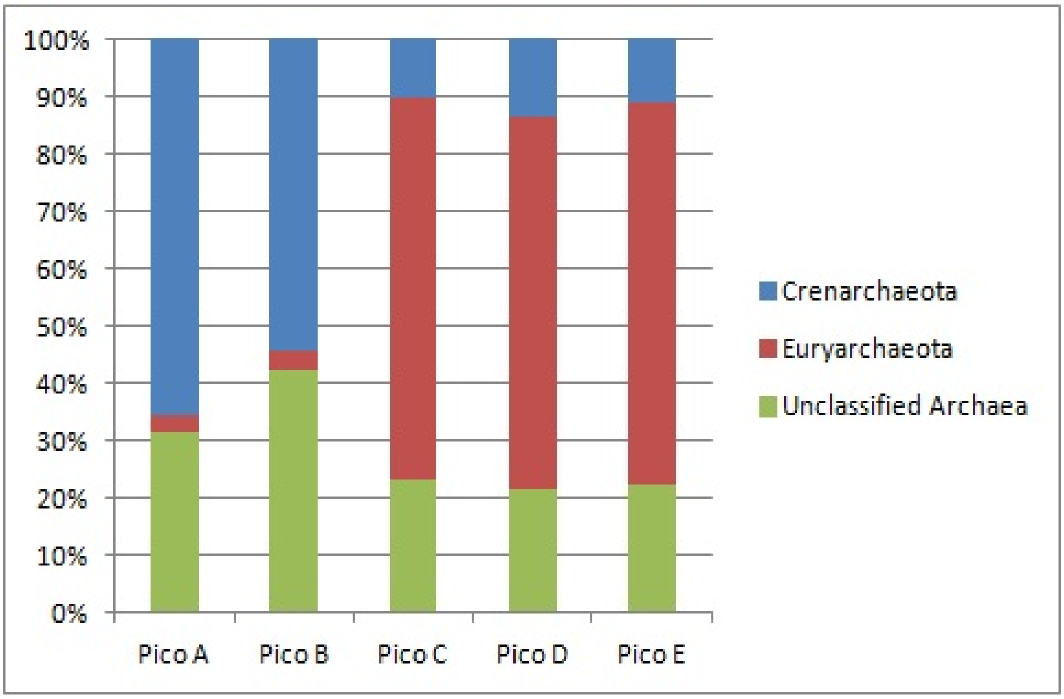
Yuste, L., et al. (2000). Characterization of bacterial strains able to grow on high molecular mass residues from crude oil processing. *FEMS Microbiol Ecol*, *32*, 69-75. http://dx.doi.org/10.1111/j.1574-6941.2000.tb00700.x

Appendix



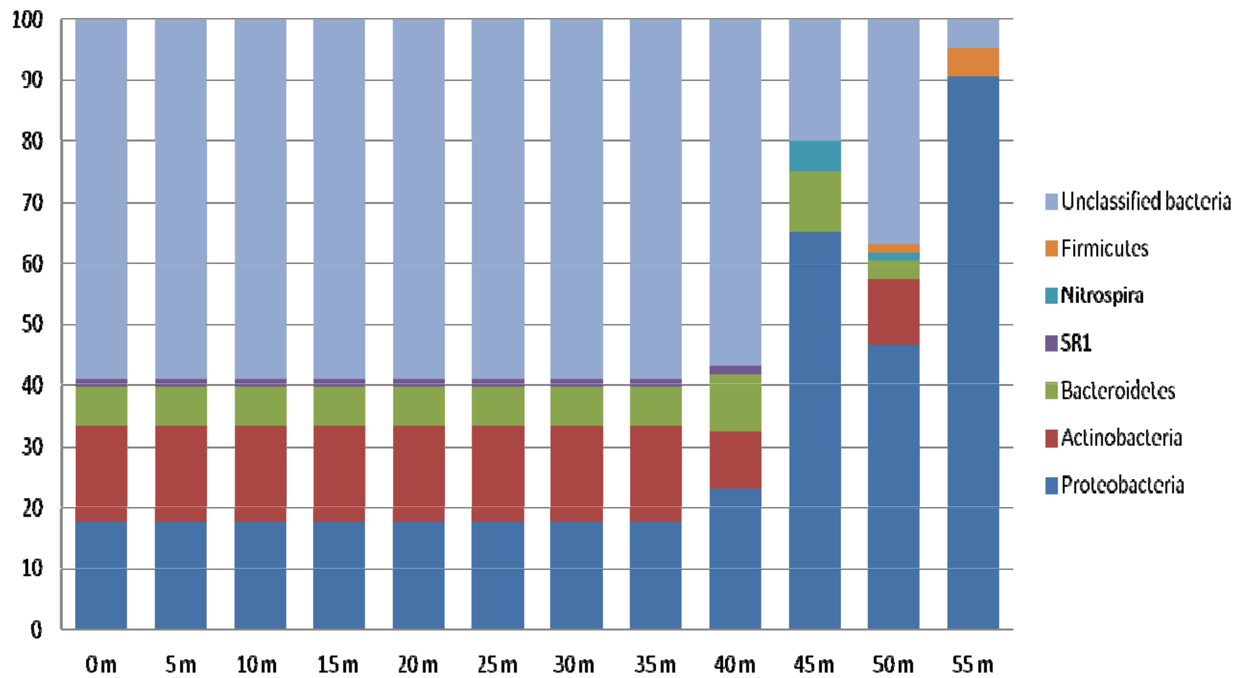
Appendix 1. Archaeal diversity by depth

Description: Histograms showing the archaeal diversity at a phylum level in each layer.



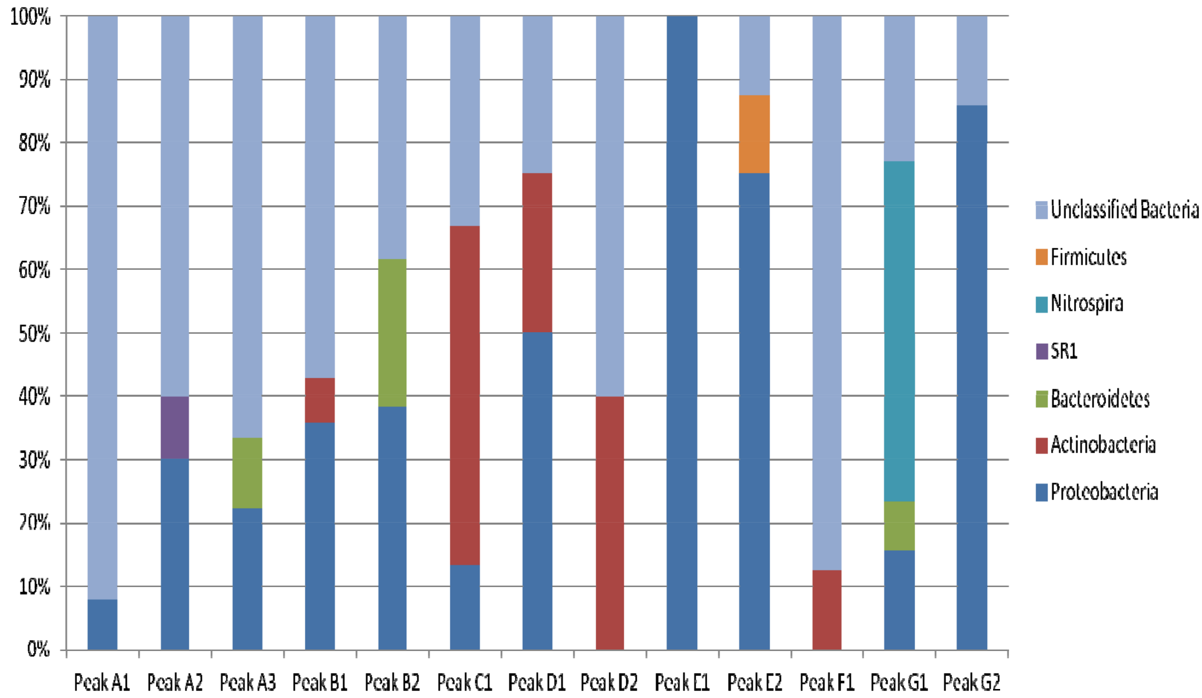
Appendix 2. Archaeal diversity by peaks

Description: Histograms showing the archaeal diversity at a phylum level in each peak.



Appendix 3. Bacterial diversity by depth

Description: Histograms showing the bacterial diversity at a phylum level in each layer.



Appendix 4. Bacterial diversity by peaks

Description: Histograms showing the bacterial diversity at a phylum level in each peak.

Copyright Disclaimer