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Running title: Active archaeal communities in lacustrine ecosystems

Keywords: *Archaea* / diversity / lakes / *amoA* / active biosphere

Summary

Deep lakes are of specific interest in the study of archaeal assemblages as chemical stratification in the water column allows niche differentiation and distinct community structure. Active archaeal community and potential nitrifiers were investigated monthly over one year by pyrosequencing 16S rRNA transcripts and genes, and by quantification of archaeal *amoA* genes in two deep lakes. Our results showed that the active archaeal community patterns of spatial and temporal distribution were different between these lakes. The meromictic lake characterized by a stable redox gradient but variability in nutrient concentrations, exhibited large temporal rearrangements of the dominant euryarchaeal phylotypes, suggesting a variety of ecological niches and dynamic archaeal communities in the hypolimnion of this lake. Conversely, *Thaumarchaeota* MGI largely dominated in the second lake where deeper water layers exhibited only short periods of complete anoxia and constant low ammonia concentrations. Investigations conducted on archaeal *amoA* transcripts abundance suggested that not all lacustrine *Thaumarchaeota* conduct the process of nitrification. A high number of 16S rRNA transcripts associated to crenarchaeal group C3 or the Miscellaneous Euryarchaeotic Group indicates the potential for these uncharacterized groups to contribute to nutrient cycling in lakes.

Introduction

Planktonic freshwater habitats have emerged as an unsuspected reservoir of archaeal diversity (Galand *et al.*, 2006; Lliros *et al.*, 2010; Auguet *et al.*, 2011) and abundance, which range from 1 to 20% of the total bacterioplankton (Pernthaler *et al.*, 1998; Glockner *et al.*, 1999; Keough *et al.*, 2003). Most studies have investigated shallow lakes (Bosshard *et al.*, 2000; Auguet and Casamayor, 2008; Auguet *et al.*, 2011) or only the upper part of lake water

column (Boucher *et al.*, 2006; Hugoni *et al.*, 2013a). In these water layers archaeal communities are often composed of *Thaumarchaeota* (Auguet *et al.*, 2011; Hugoni *et al.*, 2013a) but contrasted temporal dynamics were reported for this group with maximal abundances retrieved either in winter or in summer without any clear trend. Deep water layers remain less studied than surface ones even though they represent an important part of freshwater on Earth. In deep lakes, *Thaumarchaeota* tended to dominate in both oxycline and halocline as previously described in Lake Kivu (Lliros *et al.*, 2010) and an Arctic saline lake (Comeau *et al.*, 2012). Moreover, archaeal *amoA* gene abundance, diagnostic for potential ammonia oxidation, was associated with *Thaumarchaeota* in those ecosystems (Lliros *et al.*, 2010; Comeau *et al.*, 2012). *Thaumarchaeota* are known to be key players in the aquatic nitrogen cycle (Schleper and Nicol, 2010; Walker *et al.*, 2010), but their phylogenetic diversity and the recent view that they likely have a variety of metabolisms (Pester *et al.*, 2011; Stahl and de la Torre, 2012; Beam *et al.*, 2013) indicate that they may be adapted to a variety of niches.

Thaumarchaeota potential activity in relation to seasonal changes, salinity and chemical gradients remains poorly understood in lakes. *Thaumarchaeota* are for instance rare in deeper waters (i.e. anoxic and/or suboxic zone and above sediments) (Lliros *et al.*, 2010; Vissers *et al.*, 2013) where methanogenesis performed by *Euryarchaeota* is often the most important process (Lehours *et al.*, 2005; Lliros *et al.*, 2010). The highly diverse Miscellaneous Crenarchaeotic Group (MCG, (Inagaki *et al.*, 2003)), the uncultured Marine Benthic Group D (MBG-D, (Galand *et al.*, 2012)), or the uncultured crenarchaeal group C3 (Comeau *et al.*, 2012) have also been identified in lacustrine deeper layers, as well as *Euryarchaeota* affiliated with the Lake Dagow Sediment cluster (LDS (Glissman *et al.*, 2004)) and the Rice Cluster V (RC-V (Großkopf *et al.*, 1998)). LDS and RC-V groups are highly diverse and frequently retrieved in lakes (Jurgens *et al.*, 2000; Glissman *et al.*, 2004) and rivers (Galand *et al.*, 2006;

Herfort *et al.*, 2009), suggesting that they play a key functional role in freshwater habitats (Barberan *et al.*, 2011). However, their levels of activity are still poorly characterized in aquatic ecosystems.

Deep lakes provide ideal systems to study microbial communities associated to biogeochemical processes in stratified water bodies. High physical stability of the water masses results in relatively constant vertical stratification and transition between oxic-anoxic zones, and in many cases, the presence of a dense microbial community at the redox transition zone (Bosshard *et al.*, 2000). Moreover, the vertical physicochemical gradients retrieved in these ecosystems may provide a variety of niches for microbial growth and differentiation (Pouliot *et al.*, 2009). Although several studies have focused on lakes stratified by salinity (Bosshard *et al.*, 2000; Pouliot *et al.*, 2009; Comeau *et al.*, 2012), only a few studies have investigated lakes that were meromictic due to their important depth (Lehours *et al.*, 2005). Most studies investigating lacustrine ecosystems have focused on archaeal diversity based on gene abundance, and the potentially active archaeal assemblages remain poorly investigated (La Cono *et al.*, 2013; Vissers *et al.*, 2013). Similarly, active ammonia oxidizing *Archaea* (AOA) are rarely studied in freshwater ecosystems (Hatzenpichler, 2012). Recent studies have shown the importance of differentiating the active from the total communities (Jones and Lennon, 2010; Campbell *et al.*, 2011; Hugoni *et al.*, 2013b). One method to explore an aspect of their activity (i.e. the growth rate for specific taxa) is to investigate microbial communities at both the 16S rRNA genes and 16S rRNA transcript level (Campbell *et al.*, 2009; Lami *et al.*, 2009).

In this study, we targeted archaeal communities in the deep water layers of two lacustrine ecosystems to test the hypotheses that (i) archaeal assemblages and abundance are different in the two lakes because of contrasting environmental conditions across the water column, (ii) a stable redox gradient favors the establishment of a diverse *Archaea* community

(stability should promote niche diversification), and in contrast, an instable redox gradient (reoxygenation each year) promotes less diverse generalist microorganisms able to cope with variation in environmental conditions; (iii) different ecotypes of *Thaumarchaeota* could inhabit these under sampled ecosystems. We studied a strongly stratified lake with a stable redox gradient (Lake Pavin), and a lake with a transitory redox gradient due to mixing events through the entire water column in winter (Lake Bourget). We targeted both the oxycline and anoxic and/or suboxic zone of these two lakes by characterizing the total (16S rRNA genes) and active (16S rRNA) archaeal assemblages and quantifying *amoA* transcripts.

Results and Discussion

Physico-chemical and biological characteristics of the lakes

Lake Pavin had a permanent oxycline and anoxic zone while Lake Bourget only had temporary oxycline and anoxic conditions in the bottom of the lake (Table S1). During the entire sampling year, the oxygen concentration ranged from 1 to 2.89 mg L^{-1} in the oxycline of Lake Pavin (45 m). In contrast, Lake Bourget was characterized by an oxycline layer at 130 m, which was oxic from March to June (oxygen between 7.93 and 9.47 mg L^{-1}) and was depleted of oxygen from July to December (oxygen from 3.24 and 6.61 mg L^{-1}). Similarly, at 140 m, this lake was oxygenated from March to June (oxygen between 5.96 and 9.37 mg L^{-1}), and nearly anoxic from August to December (oxygen ranging between 0.06 and 1.34 mg L^{-1}). In Lake Bourget, the average ammonia concentrations were very low compared to Lake Pavin, but inversely there was in average more nitrate in Lake Bourget compared to Lake Pavin (Table S1). The coefficient of variation established for the different environmental parameters illustrated a large disparity in phosphate and nitrate concentrations in Lake Pavin while they were more stable in Lake Bourget. Large coefficients of variation were also seen for ammonia concentrations for the two layers in both lakes (Table S1).

Archaeal community composition

Changes in the community structure of active archaeal populations were evaluated over time in both Lake Bourget and Pavin by deep sequencing of 16S rRNA genes and transcripts (Table S2). The Chao1 index showed that richness was highest in Lake Pavin than in Lake Bourget (Figure 1). Overall the composition of active archaeal communities was significantly different (NPMANOVA, $P < 0.001$; Figure 2) between the two lakes, dominated by the phylum *Euryarchaeota* in Lake Pavin and *Thaumarchaeota* in Lake Bourget. The average number of sequences was calculated for the major taxonomic groups retrieved in each ecosystem and potential activity was inferred for each group from the 16S rRNA/16S rRNA genes ratio.

In the oxycline zone of Lake Pavin, some groups such as *Thaumarchaeota* MGI and *Euryarchaeota* LDS presented 16S rRNA/16S rRNA genes ratio <1 suggesting no or low activity, while others, such as MEG or *Methanosaeta*, had ratios >1 illustrating a greater potential activity in the anoxic zone (Figure 3). MEG dominated the active archaeal assemblage throughout the entire year, suggesting that this poorly characterized group could play a key functional role in aquatic ecosystems. These *Euryarchaeota* MEG were dominated by 4 different OTUs with best Blast match to sequences recovered from Spanish lakes (Lliros *et al.*, 2008; Auguet *et al.*, 2011), indicating the presence of a freshwater clade. Members of this group have also been retrieved in deep subsurfaces (Hirayama *et al.*, 2007), soils and marine sediments (Takai *et al.*, 2001). In the anoxic zone of Lake Pavin, *Methanosaeta* was the most active group (based on the ratio 16S rRNA/16S rRNA genes). This result is consistent with previous work suggesting that methanogenesis is the central process performed by *Archaea* in the anoxic zone of Lake Pavin (Borrel *et al.*, 2011). Additionally, our study highlighted the potential activity of understudied archaeal groups in this anoxic

zone, such as the group C3 belonging to the *Crenarchaeota*. This uncultured crenarchaeal group is often found in low-temperature terrestrial and marine habitats (DeLong and Pace, 2001) but also in deep marine sediments (Wang *et al.*, 2010) and saline lakes (Comeau *et al.*, 2012; Schneider *et al.*, 2013).

Lake Bourget was characterized by a clear dominance of *Thaumarchaeota* MGI, which was potentially active in this ecosystem (Figure 3). The 16S rRNA/16S rRNA genes ratio suggested that groups, such as LDS or MCG, were more active in the deeper zone. The LDS cluster is a highly diverse group (Barberan *et al.*, 2011) identified in rivers (Galand *et al.*, 2006; Herfort *et al.*, 2009), where they accounted for a large proportion of the archaeal cell counts (Herfort *et al.*, 2009; Restrepo-Ortiz *et al.*, 2013). MCG was one of the predominant archaeal groups obtained from marine deep subsurface sediments, but could also be retrieved in terrestrial, marine, hot and cold, surface and subsurface environments (Teske, 2006). Some studies suggested that this group might have a role in the carbon cycle (Biddle *et al.*, 2006) as well as in protein remineralization in anoxic marine sediments (Lloyd *et al.*, 2013). These results highlighted a contrasting picture between dominant and active *Archaea* in those freshwater ecosystems. Nevertheless, the measure of activity levels based on 16S rRNA/rRNA genes ratio could have been affected by the number of 16S rRNA gene copies per genome. However, to our knowledge, all available complete genomes of mesophilic *Archaea*, including representatives from *Euryarchaeota* and *Thaumarchaeota* showed only one copy of 16S rRNA genes, thus, we assume that this is also the case in the natural communities. We also supposed that growth rate can be correlated to the number of ribosomes per cell (Fegatella *et al.*, 1998; Campbell *et al.*, 2009) and the ratio 16S rRNA/16S rRNA genes can thus be used to highlight which archaeal groups were the most active in the different zones studied. Nevertheless, the use of 16S rRNA should be interpreted with caution

considering the environmental parameters and specific taxa due to some inconsistent relationships between 16S rRNA and activity (Blazewicz *et al.*, 2013).

Temporal dynamics of active archaeal communities

In Lake Bourget, archaeal 16S rRNA transcripts were dominated by *Thaumarchaeota* MGI sequences and MGI sequences abundance was stable throughout the entire sampling year (average 98.3% of the sequences) and mainly associated to low and stable concentrations of ammonia and higher temperatures in Lake Bourget (Figure S1). In Lake Pavin, potentially active taxonomic groups changed with time in both the oxycline and anoxic zones (Figure 4). In March, active archaeal communities were similar in both layers, while during the rest of the year, different archaeal communities were observed between the oxycline and the anoxic zone. In the oxycline of Lake Pavin (Figure 4), MEG 16S rRNA transcripts dominated the archaeal sequences year-round, except in March (27% of the sequences) and November (11% of the sequences). *Thaumarchaeota* MGI transcripts were especially abundant in November but also present in April and December. In the anoxic zone of Lake Pavin, *Methanosaeta* transcripts were present year-round and particularly in September (reaching 74% of the sequences). During that month, *Methanomicrobiales* transcripts reached 19% of the sequences, which suggest a full dominance of active methanogenic lineages during this period. However, transcripts from other archaeal groups such as MEG or the crenarchaeal group C3 were more abundant during winter or spring, and MGI was never retrieved in this zone. This result highlights that the anoxic zone of this meromictic lake should not be considered as particularly stable in its microbial composition. The important variability of phosphate, ammonia and nitrate concentrations in the anoxic zone of Lake Pavin could affect the dynamics of active *Archaea* in this zone (Table S1). Nevertheless, in this study we focused only on bottom-up controls while predation and viral lysis could also control the population diversity in aquatic ecosystems (Pernthaler, 2005). Indeed, viral communities have

been retrieved in the permanently anoxic monimolimnion of Lake Pavin and could thus significantly contribute to the regulation of prokaryotic communities (Colombet *et al.*, 2009). Therefore, we hypothesized that archaeal temporal dynamics in the anoxic zone may be a combination of large variations in nutrient concentration and viral lysis of specific taxonomic groups according to the "killing the winner" hypothesis (where winners are not necessarily the most abundant, but are the most active prokaryotic populations (Winter *et al.*, 2010)).

A NMDS analysis was conducted to visualize the temporal dynamics of potentially active archaeal communities in the oxycline and the anoxic zone of both lakes. The NMDS tridimensional ordination diagram had a stress value of 0.059, which indicates a reliable representation of the original similarity matrix. The ordination diagram showed that the archaeal community structure in Lake Pavin was considerably more variable (i.e. dynamic) than the community structure in Lake Bourget (Figure 2). The statistical significance of these differences was confirmed with a NPMANOVA test ($F = 3.36E4$, $P < 0.001$). Statistical analyses performed through the forward RDA indicated that among the parameters recorded, ammonia, temperature, phosphate and oxygen explained 53.3, 23.4, 14.3 and 6.6% of the archaeal communities' structure, respectively ($P<0.05$). This indicated that nitrogen compounds seemed to be the most structuring chemical parameter among the ones we measured. The RDA plot highlighted a clear difference between the major taxonomic groups associated with Lake Bourget and those retrieved in Lake Pavin (Figure S1). Among the significant parameters recorded with the forward RDA, temperature and oxygen were mostly linked to MGI transcripts and to Lake Bourget, while ammonia and phosphate were linked to different euryarchaeal phylotypes in Lake Pavin.

Dynamics of archaeal and bacterial *amoA* transcripts

In the oxycline zone of Lake Pavin, *Thaumarchaeota* AOA transcript numbers were low while ammonia oxidizing *Bacteria* (AOB) transcripts were more abundant and retrieved year-round (Figure 5). The dominance of bacterial over archaeal nitrifiers transcripts in Lake Pavin, where ammonia concentrations were higher, is consistent with the hypothesis that low-ammonia concentrations would be more favourable for AOA activity if AOA and AOB compete for ammonia oxidation (Schleper and Nicol, 2010; Hatzenpichler, 2012). However, additional environmental parameters need to be examined to explain AOA distribution and activity (Erguder *et al.*, 2009; Hatzenpichler, 2012). Investigation of ammonia oxidizers showed that archaeal *amoA* transcript abundance was low during the entire year in Lake Bourget (less than 10 copies of transcripts.mL^{-1} from March to October) even though *Thaumarchaeota* MGI 16S rRNA sequences dominated. However, an increase in both archaeal and bacterial *amoA* transcripts were observed during the winter period when hypoxia occurred (Figure 5). Even though we should remain careful when assuming that *amoA*-carrying *Archaea* are indeed oxidizing ammonia (Pester *et al.*, 2011), this result suggests that lacustrine oxyclines are not a hotspot for archaeal ammonia oxidation and that *Thaumarchaeota* MGI are using another metabolic pathway to gain energy. Indeed, it has been proposed that these microorganisms present a large metabolic plasticity, from autotrophic to potential mixotrophic lifestyles (Ingalls *et al.*, 2006; Hansman *et al.*, 2009). Under the anoxic conditions of the deeper zone of Lake Bourget, the aerobic nitrifiers may have been replaced by anaerobic ammonium oxidation (anammox) bacteria, such as those detected in the deep anaerobic waters of the Black Sea (Kuypers *et al.*, 2003). Further molecular analyses targeting nitrifying, denitrifying and anammox *Bacteria* would be required to better identify the community involved in nitrogen cycling. In the anoxic zone of both lakes, no AOA or AOB *amoA* transcripts were detected (data not shown).

Overall, our work revealed different patterns of active archaeal assemblages in these two deep lakes and indicated that these microbial communities in monimolimnion is much more dynamic than previously thought. We also note that taxa present in small proportions can be overrepresented in the active fraction and may thus play key functional role in freshwater ecosystems.

Acknowledgments

Physicochemical data were analyzed by the observatory on peri-alpine lakes (Database SOERE OLA INRA, UMR CARRTEL, Thonon les Bains, and data CISALB). We thank Allenvi for their support to the observatory on peri-alpine lakes (including monitoring on microbial diversity). We thank G. Paolini, P. Perney, L. Lainé and L. Jaccas for their technical contributions to sampling, analysis of physical and chemical parameters in Lake Bourget, and contribution to water samples' preparation. We thank S. Palesse, M. Charpin and J. Colombet for their support on the field in Lake Pavin, G. Bronner, E. Duffaud, A. Vellet, A. Moné and I. Louati for physical and chemical analyses, and contribution to molecular analyses. PEG is supported by the Agence Nationale de la Recherche (ANR) through the MICADO project (ANR-11-JSV7-003-01).

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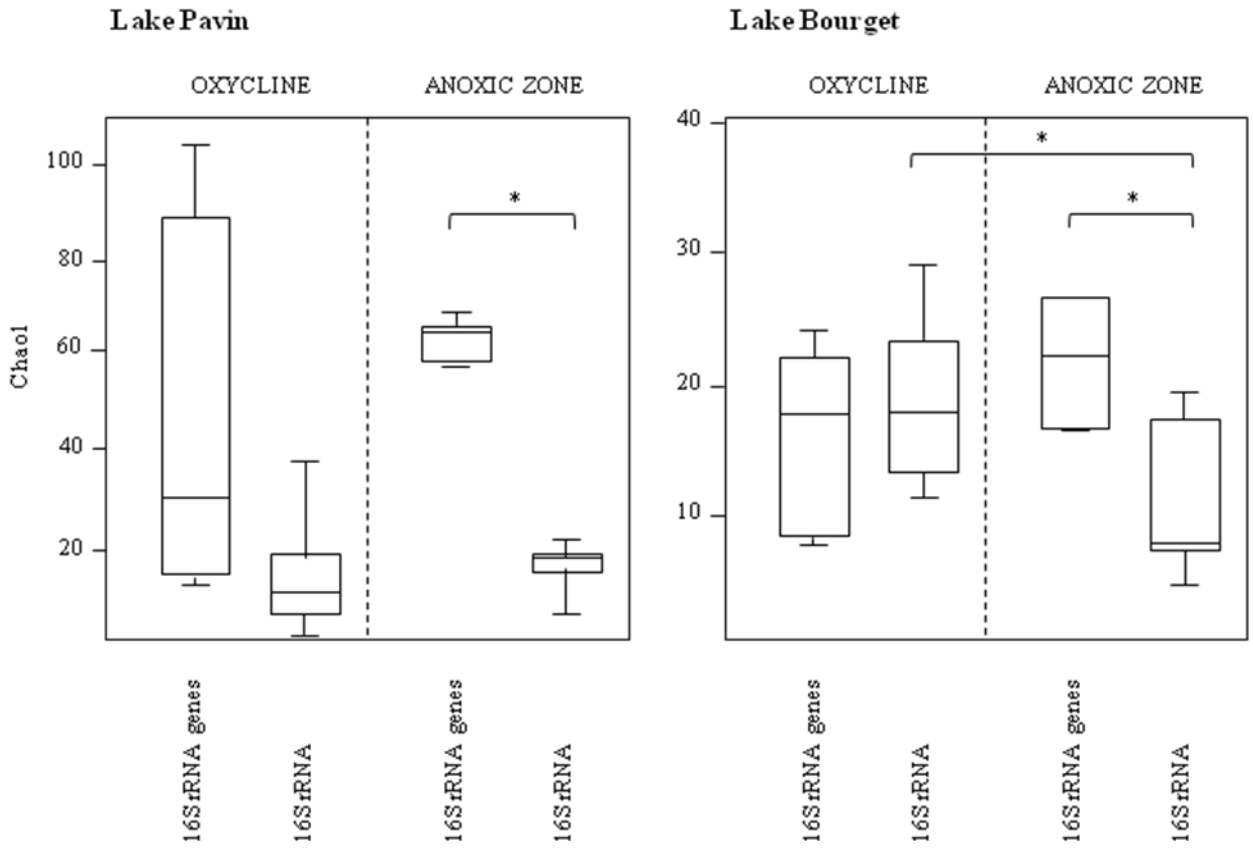

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Figure 1. Boxplot of the Chao1 index in the oxycline and the anoxic zones of both lakes. A significant difference in richness among the lakes is marked with a star (*, $p < 0.05$).

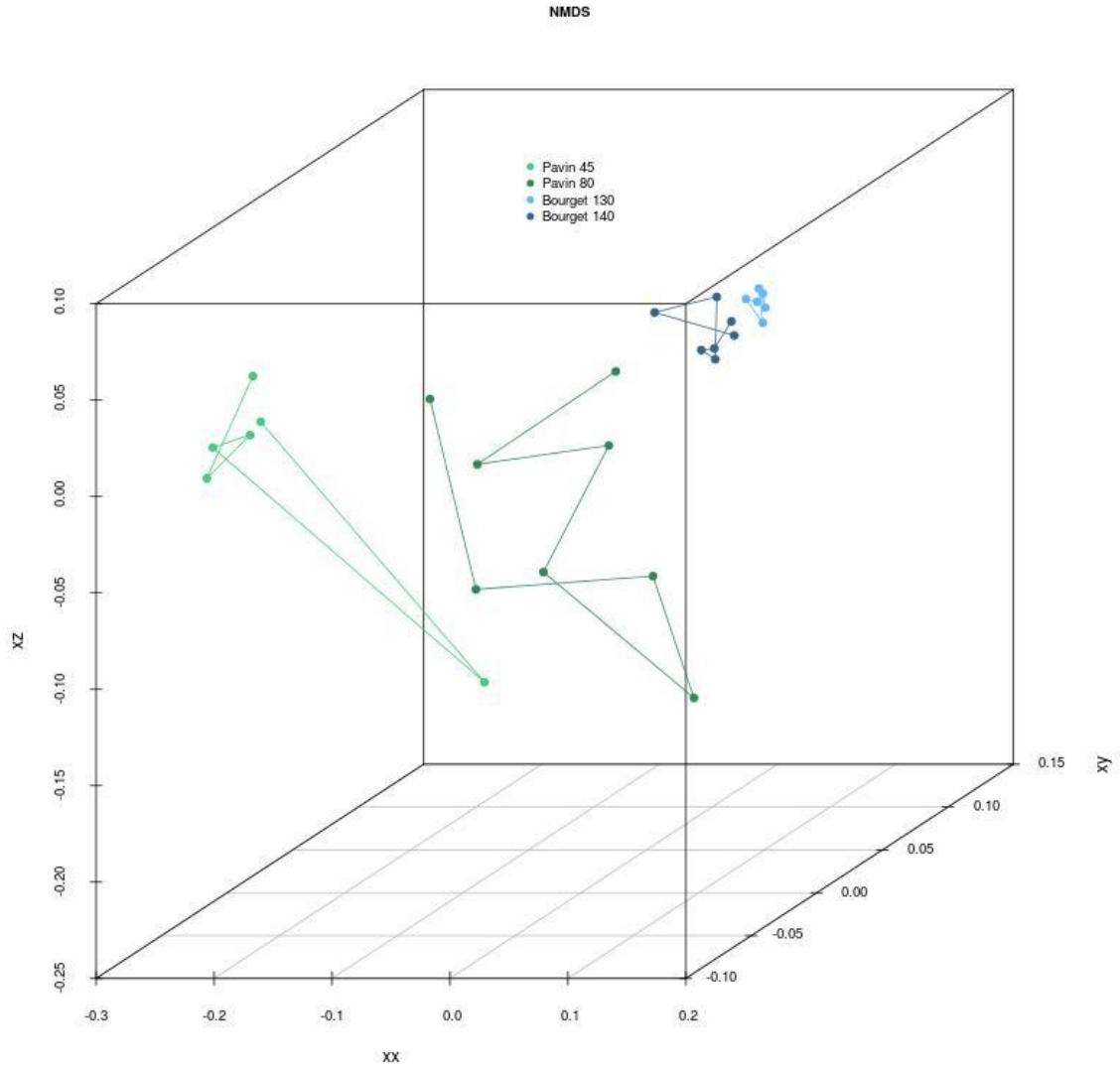

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Figure 2. Non-Metric Multi-Dimensional Scaling (NMDS) ordination diagram of temporal variations in archaeal communities' structure. The ordination is based on a Bray-Curtis similarity matrix of the square root transformed abundance data obtained from the sequences counts in both lakes. A One-way Non-Parametric Multivariate Analysis of Variances
485 (NPMANOVA: F = 3.36E4, P< 0.001) was calculated to test the significance of the differences observed in the NMDS ordination plot.

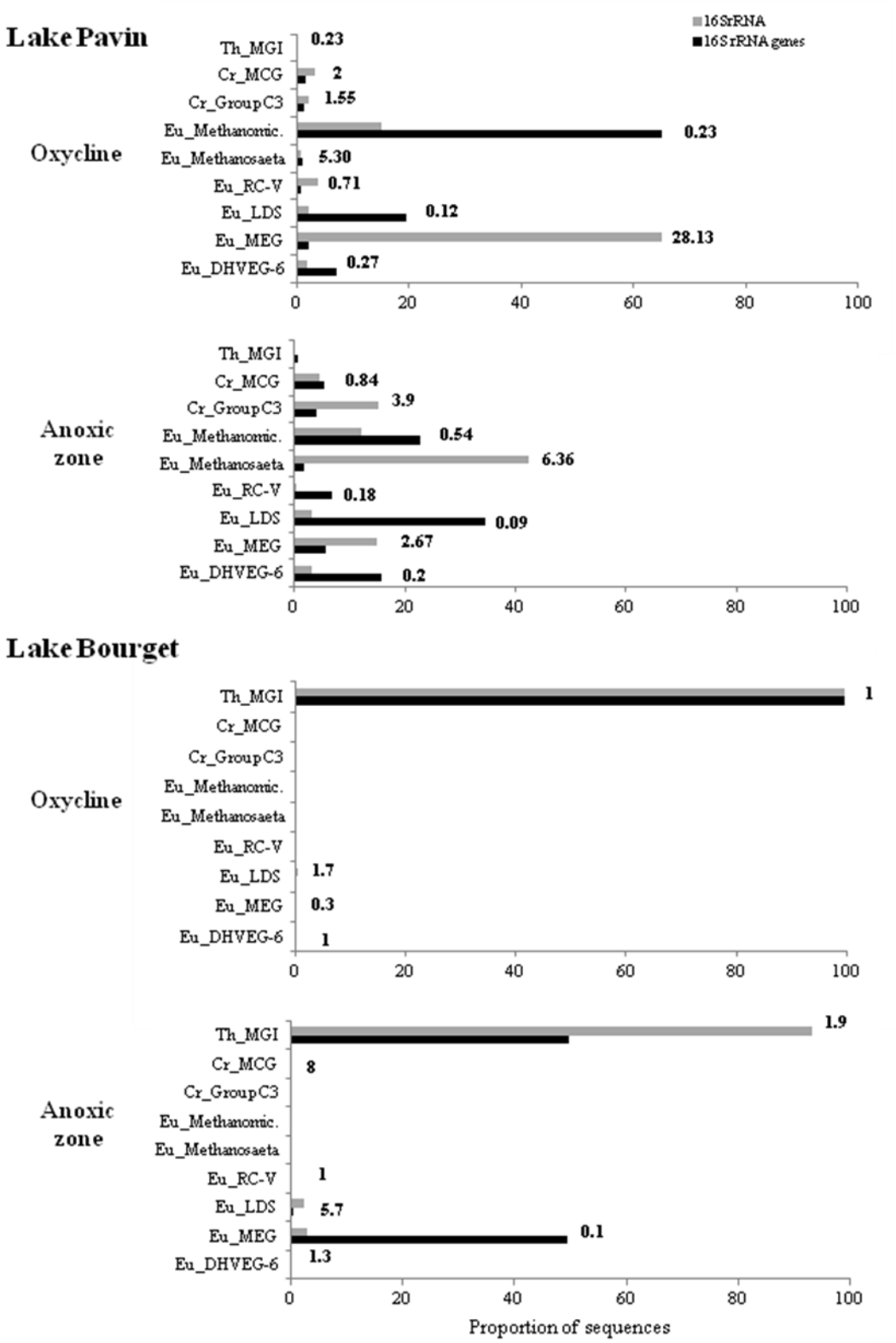

Figure 3. Number of sequences affiliated with the main archaeal groups retrieved in both the 16S rRNA genes and 16S rRNA datasets of Lake Pavin and Lake Bourget. Bold values indicate the 16S rRNA/16S rRNA genes ratios. Eu: *Euryarchaeota*, Cr: *Crenarchaeota*, Th: *Thaumarchaeota*, Methanomic.: *Methanomicrobiales*.

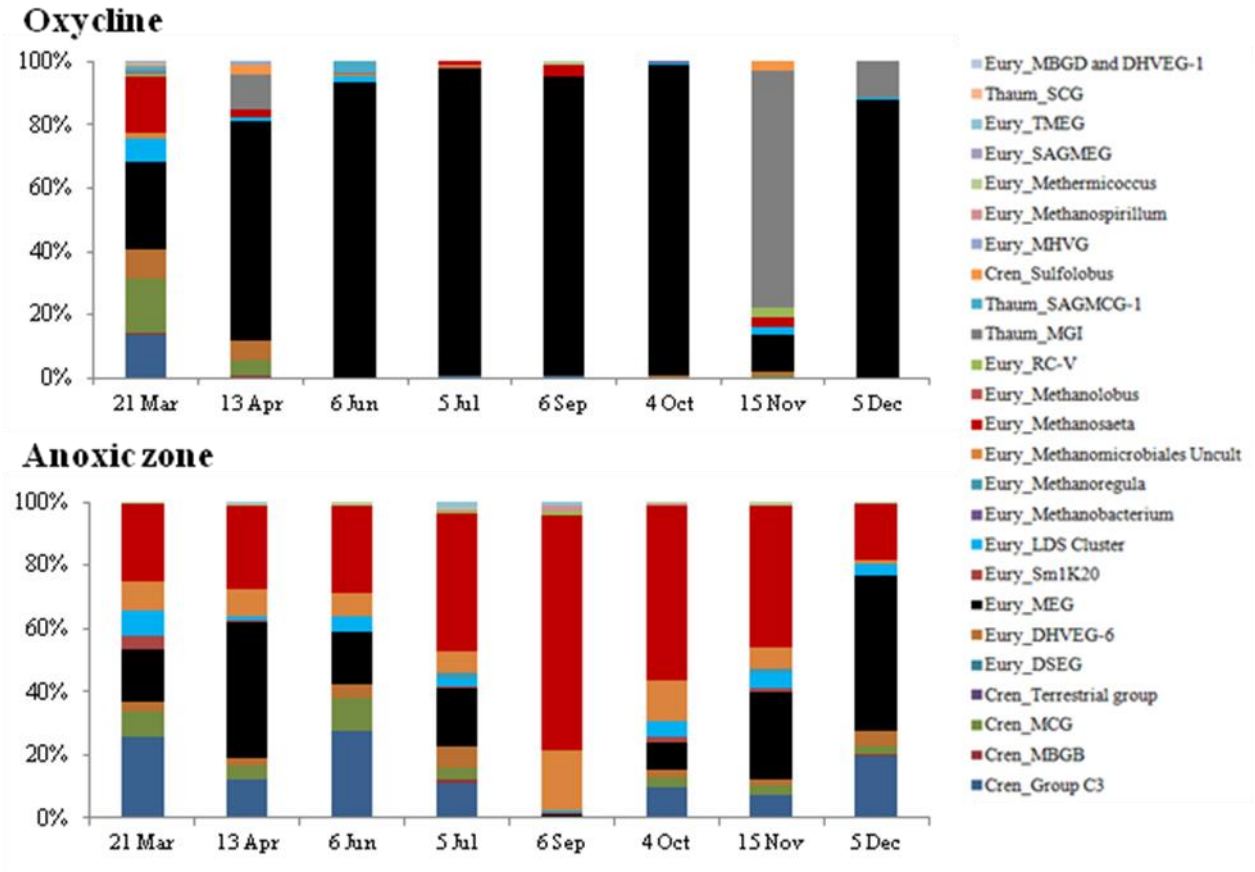

Figure 4. Relative 16S rRNA transcripts abundance of active archaeal groups in Lake Pavin oxycline and anoxic zones. Eury: *Euryarchaeota*, Cren: *Crenarchaeota*, Thaum: *Thaumarchaeota*.

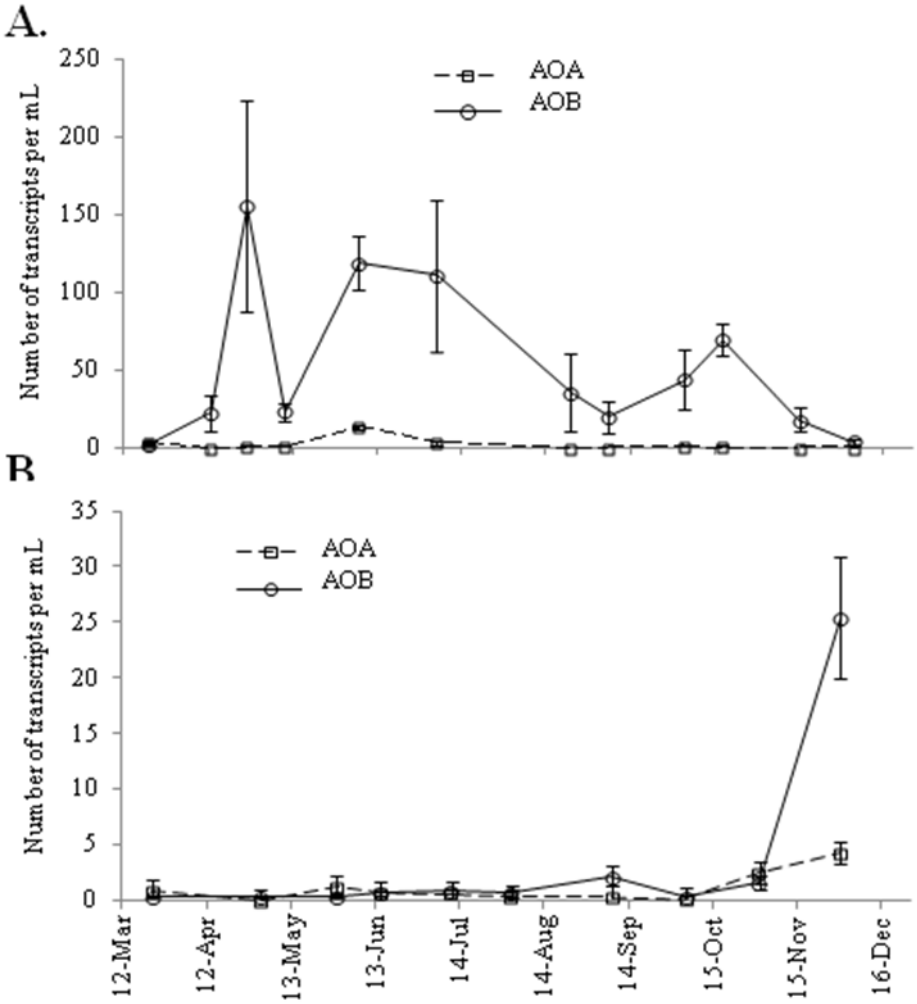

Figure 5. Archaeal and bacterial *amoA* transcript abundance over one year in the oxycline zone of Lake Pavin (A) and Lake Bourget (B).

Supporting Information

Materials and Methods

Study sites, sampling and environmental parameters

This study was performed on two lakes located in France: Lake Pavin in the Massif Central, and Lake Bourget at the edge of the Alps. Lake Pavin (45°55'N; 2°54'E) is a meromictic, oligomesotrophic freshwater lake, situated at an altitude of 1197 m, with a maximal depth of 92 m. It is fed by atmospheric precipitations and numerous superficial and sub-lacustrian springs (Viollier *et al.*, 1997). Lake Bourget (45°44'N; 5°51'E) is a freshwater lake, situated at an altitude of 231 m, and where mixing events in the whole water column (maximal depth of 145.5 m) has occurred frequently within recent years (observatory on deep peri-alpine lakes, INRA Thonon Les Bains). This lake presented an oxic-anoxic transition zone and a deeper layer, which is anoxic and/or suboxic for several months of the year.

During 2011, water samples were collected monthly in the oxycline layer (at 45 m and 130 m for Lake Pavin and Bourget, respectively, during thermal stratification), and anoxic and/or suboxic zones (80 m and 140 m for Lake Pavin and Lake Bourget, respectively) using a Van Dorn bottle at a permanent station located at the deepest zone of the water column. The water temperature and dissolved oxygen content were determined using a multiparameter probe (YSI GRANT 3800). The phosphorus ($P\text{-}PO_4$), nitrate ($N\text{-}NO_3$) and ammonium ($N\text{-}NH_4$) contents were analyzed using standard American Public Health Association 100 (1992) methods. The chlorophyll-*a* (Chl*a*) content was determined using spectrophotometry (Lorenzen, 1967).

Nucleic acids extraction and pyrosequencing

A sub-sample of water (300 mL) added with an equal volume of RNA Later (ammonia sulfate 7.93 M, sodium citrate 0.025 M, EDTA 0.02 M qsp 1.5 L of RNAse free water, pH 5.2), was pre-filtered through 5-µm pore-size polycarbonate filters (Millipore) and collected on 0.2-µm pore-size (pressure <10 kPa) polycarbonate filters (Millipore) before storage at –80°C until nucleic acid extraction. The nucleic acids extraction method was modified from Hugoni *et al.* (2013) using a combination of mechanical and enzymatic cell lysis, followed by extraction using the AllPrep DNA/RNA kit (Qiagen, Valencia, CA). RNA samples were tested for the presence of contaminating genomic DNA using PCR and then reverse transcribed with random primers using SuperScript® VILO (Invitrogen). Amplification of the V4-V5 region of the 16S rRNA genes and 16S rRNA cDNA was performed using the universal archaeal primers Arch519F (Herfort *et al.*, 2009) and Arch915R (Casamayor *et al.*, 2002) (Supplementary Table 2). Pyrosequencing was achieved by the GINA Platform (Clermont-Ferrand, France), using a Roche 454 GS-FLX system with titanium chemistry.

Bioinformatic analyses

Pyrosequencing data for both 16S rRNA genes and 16S rRNA datasets represented 698,901 raw sequences. Cleaning procedures consisted in the elimination of sequences presenting ambiguous bases "N", a quality score < 25, length shorter 200pb and with a mismatch in the forward primer. The remaining sequences were clustered at a 97% similarity threshold (Kim *et al.*, 2011) and representative sequence for each OTU were inserted in phylogenetic trees for taxonomic annotation. This process was automated by PANAM that also computed richness and diversity indexes, Chao1 and Shannon respectively (http://code.google.com/p/panam-phylogenetic-annotation /downloads /list) (Taib *et al.*, 2013). Chimeras were detected using Uchime (Edgar *et al.*, 2011) and represented 0.8% of the cleaned sequences. After the removal of sequences affiliated with *Bacteria*, the dataset contained a total of 104,675

archaeal sequences for the 16S rRNA genes dataset and 117,913 sequences for the 16S rRNA dataset. Many sequences were affiliated with *Bacteria* suggesting that the chosen *Archaea* primers were not as specific as thought, and that they may not have amplified all archaeal sequences in the two lakes considered. To compare 16S rRNA genes and 16S rRNA datasets, samples were randomly resampled down to 223 and 1247 sequences for Lake Pavin and Bourget, respectively.

The pyrosequencing data reported in this paper has been deposited in the MG-RAST database, http://metagenomics.anl.gov/linkin.cgi?project=10561.

Statistical analyses on sequencing data

Community structure was analysed using a matrice that was square-root transformed to minimize the impact of highly dominant OTUs and then subject to statistical analyses to compare the structure of the archaeal communities within and between both lakes. The dynamics of archaeal communities were primarily analyzed by non-metric multidimensional scaling (NMDS). A stress value was calculated to measure the difference between the ranks on the ordination configuration and the ranks in the original similarity matrix for each repetition (Ramette, 2007). An acceptable stress value should be below 0.1. Non-parametric multivariate analysis of variance (NPMANOVA) was conducted to test the differences in overall archaeal community composition between the lakes and to further confirm the results observed in the NMDS plot. All analyses were based on similarity matrices calculated with the Bray-Curtis similarity index.

To explain the temporal variation of archaeal community structure, redundancy analysis (RDA) was used after a forward selection (Borcard *et al.*, 1992) of the environmental variables (temperature, oxygen, phosphate, and ammonia concentrations) explaining a significant part of changes in the archaeal taxonomic clusters abundance (inferred from

sequences number). This analysis was performed with the VEGAN package (http://cran.r-project.org/web/packages/vegan/index.html) in R.

Quantitative PCR analysis

The qPCR protocol was modified from (Hugoni *et al.*, 2013)) and used to quantify archaeal 16S rRNA genes and transcripts, and bacterial and thaumarchaeal *amoA* transcripts. The reaction mixture (25 µL) contained MESA GREEN qPCR MasterMix Plus for SYBR Assay® (1X, Eurogentec) added with 0.8 µg of BSA, 0.7 µM of primers (Supplementary Table 2) and ultra-pure sterile water. One µL of nucleic acids was added to 24 µL of mix in each well. All qPCR reactions were performed in triplicate and consisted of an initial denaturing step at 94°C (for 15 min for thaumarchaeal *amoA*, and 5 min for archaeal 16S rRNA genes and bacterial *amoA* genes) and followed by 40 cycles (thaumarchaeal *amoA*: 94°C 15 sec, 52°C 30 sec, 72°C 30 sec; bacterial *amoA*: 95°C 30 sec, 56°C 40 sec, 72°C 2 min; archaeal 16S rRNA genes: 94°C 30 sec, 57°C 40 sec, 72°C 40 sec). Standard curves were generated from a mix of clones that were representative of the environments studied. All reactions were performed with standard curves spanning from 10^1 to 10^8 copies per µL. The mean PCR efficiencies and correlation coefficients for the standard curves were as follows: for the thaumarchaeal *amo*A assay, 108%, $r^2 = 1.00$, for the archaeal 16S rRNA genes assay, 104%, $r^2 = 0.8$, and for the bacterial *amoA* assay, 107%, $r^2 = 1.00$.

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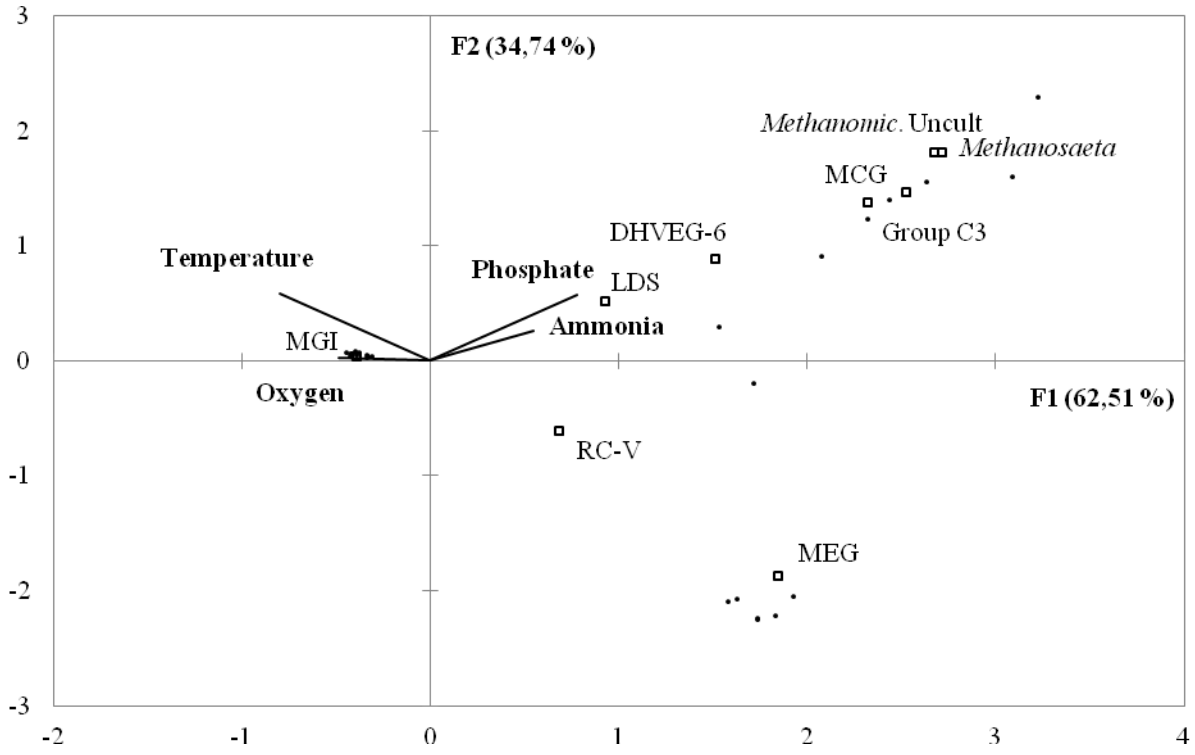
Supplementary Figures Legends



Supplementary Figure 1. Ordination diagram from RDA of the major archaeal groups compared to environmental data (temperature, oxygen, phosphate, ammonia). □: taxonomic groups, •: sampling points.

Supplementary Table 1. Environmental parameters (pH, temperature and oxygen, phosphate, nitrate and ammonia concentrations) associated with the oxycline and anoxic zones of each lake. The average values were presented, with the Min-Max values retrieved during the sampling year (9 sampling points in Lake Pavin and 12 in Lake Bourget) and the coefficient of variation (CV).

	pH	Temperature °C	Oxygen mg L^{-1}	$P\text{-}PO_4^{3-}$ mgP L^{-1}	$N\text{-}NO_3^-$ mgN L^{-1}	$N\text{-}NH_4^+$ mgN L^{-1}
Lake Pavin oxycline (45 m)						
Average values	6.81	4.23	1.73	0.05	0.11	0.42
Min-Max	6.23-7.14	4.20-4.50	1.0-2.89	0-0.09	0-0.45	0.04-0.85
CV (%)	5	2	38	238	138	70
Lake Pavin anoxic zone (80 m)						
Average values	6.22	4.70	0.43	3.3	0.16	31.21
Min-Max	5.87-7.09	4.20-5.10	0.41-0.55	0-6.10	0-0.87	5.29-98.46
CV (%)	6	9	19	58	165	115
Lake Bourget oxycline (130 m)						
Average values	7.49	5.48	6.15	0.03	0.56	0
Min-Max	7.34-7.86	5.47-5.50	3.24-9.47	0.01-0.04	0.45-0.64	0-0.008
CV (%)	2	0	35	37	14	64
Lake Bourget anoxic zone (140 m)						
Average values	7.34	5.50	2.82	0.04	0.55	0.09
Min-Max	7.18-7.85	5.43-5.52	0.06-9.37	0.01-0.06	0.47-0.75	0.001-0.24
CV (%)	3	1	122	49	10	135

Supplementary Table 2. Primers used for pyrosequencing and RT-qPCR in this study.

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Application	Primer	Primer sequence 5' – 3'	Annealing temperature	Targeted gene	Reference
Pyrosequencing	Arch519F	CAGCCGCCGCGGTAA	57°C	Archaeal 16S rRNA	Herfort *et al.*, 2009
	Arch915R	GTGCTCCCCCGCCAATTCCT			Casamayor *et al.*, 2002
qPCR	CrenAmoAModF	TGGCTAAGACGMTGTA	52°C	Thaumarchaeal *amoA*	Mincer *et al.*, 2007
	CrenAmoAModR	AAGCGGCCATCCATCTGTA			
	amoA-1F	GGGGTTTCTACTGGTGGT	56°C	β-proteobacterial *amoA*	Rotthauwe *et al.*,1997
	AmoA-RNEW	CCCCTCBGSAAAVCCTTCTTC			