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### **published in**

FEMS Microbiology Ecology  
2013

### **DOI (link to publisher)**

[10.1111/1574-6941.12013](https://doi.org/10.1111/1574-6941.12013)

### **document version**

Peer reviewed version

[Link to publication in KNAW Research Portal](#)

### **citation for published version (APA)**

Vissers, E. W., Blaga, C. I., Bodelier, P. L. E., Muyzer, G., Schleper, C., Sinninghe Damsté, J. S., Tourna, M., & Laanbroek, H. J. (2013). Seasonal and vertical distribution of putative ammonia-oxidizing thaumarchaeotal communities in an oligotrophic lake. *FEMS Microbiology Ecology*, 83(2), 515-526. <https://doi.org/10.1111/1574-6941.12013>

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**Seasonal and vertical distribution of putative ammonia-oxidizing  
thaumarchaeotal populations in an oligotrophic lake**

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**Running title:** Seasonal distribution of freshwater thaumarchaeota

20 **Subject category:** Microbial population and community ecology

**Keywords:** *amoA* / Archaea / AOA / freshwater lake / nitrification / Thaumarchaeota

## **Abstract**

The discovery of Archaea carrying an *amoA* gene coding for the A-subunit of ammonia monooxygenase gave a boost to studies aimed at detecting this gene under diverse conditions. Despite numerous studies describing the archaeal *amoA* gene abundance and richness in different habitats, the understanding of the freshwater ecology of potentially  
5 archaeal ammonia-oxidizers, recently positioned in the phylum Thaumarchaeota, is still lacking.

In a seasonal and vertical study of deep oligotrophic Lake Lucerne, Switzerland, with high thaumarchaeota-specific crenarchaeol concentrations, we show that all archaeal  
10 16S rRNA genes found belong to the thaumarchaeotal phylum. The abundances of archaeal 16S rRNA and *amoA* genes remained in the same order of magnitude (average  $6 \times 10^4 \text{ ml}^{-1}$ ) and displayed matching seasonal dynamics within sixteen monthly collected samples at three different water depths ( $r^2=0.80$ , slope of 1.06).

The thaumarchaeota in this lake form a distinct cluster in both the 16S rRNA and *amoA*  
15 gene phylogenies, are affiliated to other thaumarchaeotal freshwater sequences within the group 1.1a Archaea and fall in the low saline cluster of the *amoA* phylogeny.

In accordance with temperature and conductivity, the thaumarchaeota in the surface water showed a significantly different seasonality and lower abundance than their counterparts in the deeper waters. This study indicates that the ecology of  
20 thaumarchaeota, with their potential in nitrogen cycling, may differ per water depth in freshwater ecosystems.

## Introduction

The global nitrogen cycle plays an important role in driving and maintaining life on Earth. Intensified use of fertilizers and nitrogenous precipitation derived from industry and traffic has led to nitrogen enrichment in many ecosystems and a concomitant

5 eutrophication of terrestrial and aquatic habitats, global acidification and stratospheric carbon loss (*cf.* Gruber & Galloway, 2008). The amounts of reactive nitrogen in terrestrial and aquatic systems are determined by the balance between natural and anthropogenic nitrogen fixation on one side and denitrification on the other side.

Recently, it has been discovered that the process of Anammox, in which ammonium and

10 nitrite are anaerobically converted to  $N_2$  is also an important sink for reactive nitrogen (Kuypers et al., 2003). Nitrification, in which ammonia is converted to nitrite and subsequently to nitrate, connects the nitrogen fixation, denitrification and anammox processes. Representatives of the bacterial domain have until recently been considered to be the microorganisms solely responsible for nitrification. A major discovery was the

15 potential role of Archaea in the nitrification process, in silico in a metagenome study and later in the first cultivated thaumarchaeum (Konneke et al., 2005; Treusch et al., 2005). Based on their 16S rRNA gene, the first environmental clones from temperate environments were thought to belong to the phylum Crenarchaeota (DeLong, 1992; Fuhrman et al., 1992). However, Brochier-Armanet et al. (2008) proposed the new

20 phylum Thaumarchaeota based on the comparison of a set of 53 ribosomal proteins shared by Archaea and Eukarya. The availability of the full genomes of *Cenarchaeum symbiosum*, *Nitrosopumilus maritimus* and *Nitrososphaera gargensis* confirms a separate ammonia-oxidizing phylum of the Archaea, the thaumarchaeota (Spang et al., 2010). The use of archaeal *amoA* gene-based techniques made it possible to recognize *amoA*-

25 carrying archaea (e.g. Treusch et al., 2004; Francis et al., 2005; Wuchter et al., 2006; Nicol et al., 2008; Tourna et al., 2008) and the cultivation of a few representatives has proven their ability to grow on ammonia as an energy source and to oxidize it to nitrite (Konneke et al., 2005; de la Torre et al., 2008; Hatzenpichler et al., 2008; Tourna et al., 2011). Furthermore, numerous studies have shown predominance of archaea carrying

the *amoA* gene in marine and terrestrial ecosystems (e.g. Leininger et al., 2006; Wuchter et al., 2006), acknowledging that our understanding of nitrification is far from complete and that archaea might play a major role in nitrification in diverse environments (e.g. Prosser & Nicol, 2008; Martens-Habbena et al., 2009; Schleper & Nicol, 2010). Surprisingly, studies aiming at ammonia-oxidizing archaea in freshwater ecosystems are relatively scarce (Pouliot et al., 2009; Ye et al., 2009; Lliros et al., 2010; Auguet et al., 2011), although freshwater habitats are described to play a major role in the conversion of reactive nitrogen compounds to gaseous products before they reach the ocean (Seitzinger et al., 2006).

Most studies have used either the 16S rRNA gene or the *amoA* gene for the detection of ammonia-oxidizing archaea in the environment. Relatively few studies have used both genes simultaneously. In the marine realm, Beman et al. (2010), Mincer et al. (2007) and Wuchter et al. (2006) observed similar copy numbers of these genes throughout their seasonal studies on the distribution of archaea suggesting a role of most archaea in ammonia oxidation. Also in freshwater systems, both genes have been used simultaneously to detect potentially ammonia-oxidizing archaea, either seasonally in the surface water or at one time point throughout the water column (Pouliot et al., 2009; Lliros et al., 2010; Auguet et al., 2011). However, the results were less clear with respect to their potential role in the nitrogen cycle as the pool sizes of the 16S rRNA and *amoA* genes were not similar with respect to time and water depth.

Here we present a freshwater study in which archaeal 16S rRNA and *amoA* genes are quantitatively determined and identified during a period of 16 months at three different water depths of Lake Lucerne, Switzerland. Our study aimed at determining the seasonal and vertical distribution of potentially ammonia-oxidizing archaea in this deep, oligotrophic lake and determining their ecological importance as derived from their relation with environmental factors.

## Materials and methods

### *Location description*

Lake Lucerne is located in Central Switzerland at the northern alpine front, with a catchment area of 2124 km<sup>2</sup>. It stretches an area of 116 km<sup>2</sup>, contains seven basins and is fed by four major alpine rivers, providing ~80% of the lakes total water supply

5 (Schnellmann et al., 2002). The lake has a 3.4 years residence time. As an oligomictic lake a complete overturn occurs on average every six years.

The oligotrophic structure of the lake is characterized by an oxygenated water column with relatively high N concentrations (Buhner & Ambuhl, 2001; Bürgi & Stadelmann, 2002), mainly in the form of nitrate (average of 63 µmol l<sup>-1</sup>).

10

### *Sampling and DNA isolation*

Sampling was performed above a sub-aquatic hill in the middle of the Chrüztrichter Basin, (47°03'04"N, 8°35'26"E) of Lake Lucerne where water depth was 110 m.

15 Monthly, one and a half liter of lake water was collected and filtered (0.2 µm pore-sized cellulose-membrane filters) from the water surface (t = top), the middle of the water column (m = middle, 42 m depth) and just above the sediment (b = bottom, varying from 72 m to 101 m depth due to slight location changes at different sampling times and the bathymetry at the sampling point) of Lake Lucerne from January 2008 – April 2009. Filters were stored immediately at -20 °C until the moment of DNA isolation.

20 Samples for lipid analyses were taken at the same time and location with an *in situ* pump from the surface water and from 42 m depth and were subsequently analyzed as described by Blaga et al. (2011). Conductivity, temperature, oxygen and pH were measured throughout the water column with a CTD scanner.

DNA was isolated as described previously (Vissers et al., 2009).

25

### *Quantitative PCR (qPCR)*

qPCR of thaum- and crenarchaeal 16S rRNA and archaeal *amoA* genes was performed in a 20 µl mixture of 10 µl iQTM SYBR® Green Supermix (Bio-Rad), 1 µM of forward and reverse primers and 0.2 mg ml<sup>-1</sup> BSA. For standards, serial dilutions of the linearized

fosmid clone 54d9 were used (Treusch et al., 2005). For the archaeal *amoA* gene the forward primer 104(L)F (5´-GCAGGWGAYTACATYTTCTA-3´) was designed after alignment of many soil, marine and freshwater clone sequences (Tourna et al., 2011) and was modified including primer degeneracies matching clone sequences obtained from Lake Lucerne. Thus the primer should be considered specific for *amoA* gene sequences dominating this lake. Primer 616R (Tourna et al., 2008) was used as the reverse primer for the archaeal *amoA* gene. The efficiency of this primer pair was 92%. For thaum- and crenarchaeal 16S rRNA, primer pair Cren771F and Cren957R (Ochsenreiter et al., 2003) was used with an efficiency of 95%. This primer pair was chosen by its fit on freshwater archaeal sequences obtained from Lake Lucerne. Amplifications were performed in Mastercycler® ep realplex (Eppendorf). Melting curve analyses were performed at the end of every qPCR run to confirm the amplification of the target products only, followed by standard agarose gel electrophoresis for affirmation. All qPCR data presented were averaged from triplicate independent qPCR amplifications.

#### *PCR DGGE analysis*

PCR DGGE of cren- and thaumarchaeotal 16S rRNA combined was performed by a nested PCR with primer-pairs 21F-958R (DeLong, 1992) and Parch519F-Arch915R (Coolen et al., 2004) as previously described (Vissers et al., 2009). For the analysis of archaeal *amoA*, the primer set Arch-amoAF and Arch-amoAR was used as described by Wuchter et al. (2006). All DGGE gels electrophoresis were run at 100V for 19.5 hours. Bands were excised, reamplified and run on DGGE to confirm unique band formation.

#### *Clone library*

Clone libraries of the *amoA* and 16S rRNA genes were made of the water samples of 42m depth and just above the sediment taken in December. In these samples the number of representative DGGE bands and copy numbers were most abundant for both genes. Hundred clones were processed and analyzed of each sample for each gene. DNA

for the construction of the clone libraries was amplified with the general archaeal primer set 21-958 (DeLong, 1992) for 16S rRNA and with the primer set Arch-amoAF - Arch-amoAR (Francis et al., 2005) for *amoA*; both were cloned with the pGEM<sup>®</sup>-T Vector system (Promega, Madison, WI, USA). Selected clones were sequenced with their  
5 amplification primers (Macrogen Inc., South Korea).

#### *Statistical analysis and phylogenetic tree-building*

All statistical analysis was performed using the Statistica 9 program (Statsoft Inc., Tulsa, OK). A table of Spearman rank order correlations with pairwise deletion was produced of  
10 all variables, as well as a linear regression on the abundance of both genes. The effect of depth on abundance and richness of both genes was analyzed using a one-way ANOVA. Multiple regression analysis was performed to search for a correlation with a combination of environmental factors and archaeal numbers.

ARB was used for phylogenetic tree building (Ludwig et al., 2004). For 16S rRNA an  
15 alignment was made and a tree was calculated using ARB neighbor-joining with Jukes-Cantor correction. Bootstrap values for 1000 replicates were calculated using the MEGA software package (Tamura et al., 2007). For the *amoA* gene, the retrieved DNA sequences were aligned to published *amoA* gene sequences and transformed into protein sequences, after which a protein tree was created with ARB neighbor-joining, including a  
20 Kimura correction.

The number of OTUs was defined using a distance level of 3% by using the furthest neighbor algorithm in MOTHUR (Schloss et al., 2009) for both 16S rRNA and translated *amoA* gene.

#### 25 *Nucleotide sequence accession numbers*

The gene sequences reported in this study have been deposited in GenBank under accession numbers JN18643-JN18834.

## **Results**



Environmental parameters (Supplemental Figure 3)

Water was sampled monthly for 16 months at three water depths of the Chrüztrichter basin, Lake Lucerne. During the first four months of the sampling period, the water temperature and conductivity in Lake Lucerne were rather constant at 5°C and 140 - 150  $\mu\text{S cm}^{-1}$  at all sampling depths. Whereas these parameters overall remained stable in the deeper water layers throughout the sampling period, they increased rapidly in the surface layer after April 2008 to reach a temperature above 20°C and a conductivity of 190  $\mu\text{S cm}^{-1}$  in May/June and declined again slowly to their starting values from September on. pH and oxygen were constant over all three depths. pH remained around 8.5 and oxygen changed with season between 9 and 13  $\text{mg l}^{-1}$  from January to March 2008 and decreased to 8-11  $\text{mg l}^{-1}$  from September until the end of the sampling period. When ammonium levels peaked, nitrate concentrations dropped. Ammonium concentrations were mostly below detection level and nitrate was generally between 40 to 50  $\mu\text{mol l}^{-1}$ . In July, at the surface and 42 m depth, a strong maximum of ammonium (10 and 12  $\mu\text{mol l}^{-1}$ ) was observed simultaneously with a minimum of nitrate (6 and 10  $\mu\text{mol/l}$ ).

Dissolved organic carbon (DOC) and nitrogen (DON) were on average between 25 and 70  $\mu\text{mol l}^{-1}$  and 50 and 65  $\mu\text{mol l}^{-1}$ , respectively. In July 2008, DOC and DON increased at all depths, while in April 2009 both showed a maximum at the surface and 42 m water depth. In December 2008, DOC and DON increased only in the water above the sediment to 363 and 227  $\mu\text{mol l}^{-1}$ , respectively.

Richness

The observed vertical and seasonal distribution of the richness of the *amoA* and the 16S rRNA genes in the water column of Lake Lucerne showed that the surface water follows a different seasonality compared with the deeper waters (Figure 1). The spring peak of richness of both genes occurs earlier in the surface water and later and more gradual in case of the winter rise. Though from January to April 2009 the genotype richness, as indicated by the number of DGGE bands, was comparable throughout the water column,

the number of genotypes, was generally lower in the surface than in the deeper waters. This was especially the case for the 16S rRNA gene and was less pronounced for the *amoA* gene; here the minimum and maximum numbers of DGGE bands varied between seven and ten, while for 16S rRNA gene this was between one and thirty-one. In the clone libraries, made of samples in which the highest numbers of DGGE bands were obtained (*i.e.* sampled in December 2008), the numbers of OTUs found for the 16S rRNA gene was 6 and for the *amoA* gene 12, both at a 97% threshold. As the richness shown by DGGE band counts was 24 for the 16S rRNA and 7 for the *amoA* gene in December 2008, the OTUs found indicate the overestimation of archaeal 16S rRNA richness by DGGE analysis. However, the increase and decrease in richness shown by DGGE is still a valuable tool to compare richness of the same gene within samples of several depths and months.

#### Abundance

The difference between surface and deeper waters was again visible by an ANOVA analysis on the size of the thaumarchaeotal community at all depths and sampling times as presented by the total thaum- and crenarchaeal 16S rRNA and archaeal *amoA* gene copy numbers. A significant different seasonality was found for the surface water by ANOVA analysis. In general the abundance in the surface water is lower and has a more pronounced minimum number in the summer than is the case for the deeper waters (Figure 1). Interestingly, the numbers of *amoA* and 16S rRNA gene copies ( $6 \times 10^4$  copies  $\text{ml}^{-1}$  on average) correlate throughout the sampling seasons and depths as indicated by the slope of 1.06 and an  $R^2=0.80$  ( $p<0.0000$ ) of the linear regression between the numbers of both genes (Figure 2). At each depth, seasonal changes in abundance for both *amoA* and 16S genes occurred simultaneously.

#### Archaeal lipids

Spearman rank order correlation analysis pointed to a significant correlation between crenarchaeol concentration and 16S rRNA and *amoA* gene abundance and richness

(Supplementary Table 1a,b). The surface layer distinguished itself once more from the layer deeper in the water column as the crenarchaeol concentration relates differently to gene copy numbers concentration at different water depths. Whereas in the surface water the crenarchaeol concentration increases when 16S rRNA gene copy numbers rise (Figure 4), the absolute concentration of crenarchaeol was larger but also more constant in relation to the 16S rRNA gene copy number at 42 m water depth.

### Phylogeny

To determine which groups of Archaea dominated the water column of Lake Lucerne, phylogenetic trees were constructed of archaeal 16S rRNA and *amoA* gene sequences obtained from clone libraries from samples taken in December 2008 at 42 m depth and above the sediment. These two samples were selected, since the set of DGGE bands detected for both genes was representative for all observed bands found in the lake throughout the year. Phylogenetic analyses of both the functional gene and the 16S rRNA gene showed a separate Lake Lucerne cluster. The Lake Lucerne group falls within the previously described low saline cluster of the *amoA* thaumarchaeota phylogeny (Figure 3a,b). Five different sub-clusters within the Lucerne cluster were detected for both 16S rRNA and *amoA* gene.

### 20 Correlations (Supplementary Table 1)

The seasonal and vertical distribution of the concentration of ammonium, DOC and DON showed no significant correlations with the DNA- or lipid-derived values in our analysis. Whereas in the middle and lower parts of the water column no significant correlation was observed between gene abundance and nitrate, a positive and significant correlation was observed between nitrate concentration and both *amoA* and 16S rRNA gene abundance in the surface waters. Additionally a significant correlation between nitrate and either 16S rRNA or *amoA* gene richness was observed in the middle and surface water, respectively (Supplementary Table 1).

Temperature and conductivity were significantly and negatively correlated with the

richness and abundance of both *amoA* and 16S rRNA gene in the top layer. In the deeper waters where temperature and conductivity remained constant, this correlation was not found.

5 Oxygen and pH were only significantly correlated to the genetic data at 42 m water depth. For oxygen this was a negative correlation, while pH was positively correlated. The strongest correlation found when combining the parameters measured in a multiple regression analysis, was the concentrations of dissolved organic nitrogen and carbon, nitrate and ammonium on the 16S rRNA and *amoA* gene abundances and richness.

## 10 **Discussion**

Here we present for the first time a seasonal and vertical analysis by independent methods on the size and the richness of the community of thaumarchaeota in a deep lake for both the phylogenetic 16S rRNA gene and the functional *amoA* gene. Although we are aware that ammonia-oxidizing archaea and bacteria may co-exist in lacustrine environments we choose to study the first group in depth as not much is known about their abundance and diversity in lakes. Seasonal analysis of archaea in a lacustrine environment until now focused only on the 16S rRNA gene (Lliros et al., 2008) or solely on the surface water of lakes (Auguet et al., 2011). Due to the eutrophic situation in the studied lake and the low numbers of Archaea detected, no correlation of abundance with nutrients or other environmental factors was detected by Lliros et al. (2008). In Lake Lucerne, multiple regression analysis of all measured explaining variables indicated that nitrate was the only variable explaining a significant part of the variation of thaumarchaeotal abundance ( $R^2 = 0.7$ ,  $p < 0.000$ ). As nitrate is the end product of the nitrification process, our data may indicate that the thaumarchaeota in Lake Lucerne contribute to nitrification.

Thaumarchaeota in Lake Lucerne

As only thaumarchaeotal cells were detected in a general archaeal clone library and since a one to one relationship was observed in Lake Lucerne between the numbers of 16S

rRNA and *amoA* genes throughout sampling depths and times, it can be concluded that all archaeal cells harbored the gene for ammonia oxidation throughout the year as well as over the whole water column. This relationship is in accordance with the finding of one copy of the *amoA* gene per cell in *Cenarchaeum symbiosium* (Hallam et al., 2006),  
5 *Nitrosopumilus maritimus* (De Corte et al., 2009), *Nitrosocaldus yellowstonii* (Gray et al., 2010) and *Nitrosoarchaeum limnia* (Blainey et al., 2011), but differs from the 2.3 copies of *amoA* gene found per 16S rRNA gene in the surface water of high alpine lakes in Spain (Auguet et al., 2011). However, since the 16S rRNA qPCR primers used in the last study detect Archaea belonging to marine group 1.1a, but not 1.1b, which are also  
10 assumed to harbor *amoA* genes, we conclude that in the lacustrine setting thaumarchaeota harbor one *amoA* gene copy per cell.

As shown by BLAST results, the primers applied for quantification of 16S rRNA gene abundance in our study were selective towards both cren- and thaumarchaeota and not only towards the thaumarchaeota with their potential ammonia-oxidizing capacity.  
15 However, since the quantity of 16S rRNA and *amoA* genes are in unison throughout season and depth, it appears that potential ammonium-oxidizing thaumarchaeota present the majority of archaea in Lake Lucerne. Additionally, the similarity in 16S rRNA- and *amoA*-based phylogenetic trees supports a potential role for thaumarchaeota in the oxidation of ammonium.

20

#### Seasonality

The changes in abundance as demonstrated by the number of gene copies measured and the changes in richness as indicated by the number of DGGE bands observed, showed largely the same dynamics (Figure 1). Apparently, as the thaumarchaeotal  
25 community increased in size, more genotypes were detected, showing a rise in richness. An increase in community size points to an increase in substrate availability or a decrease in predation or other death factors. An increase in substrate availability does not necessarily mean an increase in the amount of ammonium, but could also indicate a raise in other, as yet unknown, substrates or co-substrates to ammonium.

### Phylogenetic richness

When thaumarchaeota are considered to belong to a similar niche, the high thaumarchaeotal phylogenetic richness found monthly is remarkable. The large richness  
5 may be explained by chaotic oscillations in aquatic food webs, for example in abundances of competitors and grazers, which allow for large numbers of species occupying the same niche to coexist (Beninca et al., 2008).

The number of different 16S rRNA DGGE bands found in our study was generally larger than the number of *amoA* DGGE bands. The DGGE method is known to sometimes  
10 produce more bands per gene in the form of heteroduplexes (Myers et al., 1989). The number of OTUs found in the clone libraries of the samples with the highest richness as shown by the DGGE method (i.e. from the water samples collected in December) was just opposite to what was found in the corresponding DGGE analysis: 12 OTUs for the *amoA* gene and 6 for the 16S rRNA gene, whereas the DGGE gave maximally 7 and 24  
15 bands for these genes, respectively, in this month of sampling. The size of the richness in the 16S rRNA and *amoA* DGGE results should therefore not be mistaken as a sign for the detection of archaea that do not harbor the *amoA* gene. The higher richness in 16S rRNA compared to *amoA* gene with more or less identical abundances of both genes can be attributed to the conserved nature of the functional gene as compared to the 16S  
20 rRNA gene.

### Unique surface water

The surface water appeared to accommodate a different thaumarchaeotal community than the deeper water layers, as suggested by the different DGGE banding patterns of  
25 both genes for the surface and deeper waters (Supplementary Figures 5 and 6). A difference between the water depths was evident from an ANOVA analysis for both the gene abundance and the size of richness of the thaumarchaeotal 16S rRNA and the *amoA* genes present. The observed difference in thaumarchaeotal communities between the surface and deeper waters does not seem to be related to the stratification of the

water column, as different communities were observed in the surface water and deeper waters both during summer stratification (*i.e.* May – December) and during winter when the water column is well mixed. At the onset and the end of summer stratification in April and December, respectively, when the water of the lake overturned completely, the community of the surface waters resembled the communities of the deeper waters during the stable situations. Also the communities of the deeper waters were less rich in community members at the time of overturn, as is the case for the surface water in both the stable summer and winter situation. This was evident by the increase in richness and number of species in the surface water at the start of the mixing events of April and December 2008 (Figure 1). As the thaumarchaeotal community in the surface water of the lake was usually different from those in the deeper waters, regardless of stratification, environmental factors other than stratification cause the community in the surface water to distinguish itself from the deeper waters. As no correlation was found between the composition of the communities and concentrations of ammonium, nitrate, oxygen, nor with pH, temperature or conductivity, likely light-related factors influence the thaumarchaeotal community in this water layer. This may be through an interaction with phototrophic microorganisms competing for nutrients or directly through inhibition by UV radiation, as seen for bacterial ammonium oxidizers (Guerrero & Jones, 1996).

## 20 Lake Lucerne phylogenetic cluster

In our phylogenetic analyses of Archaea in oligomictic Lake Lucerne, we found a phylogenetic cluster of thaumarchaeota distinct from other Archaea of the *amoA* freshwater cluster, as proposed by Francis et al. (2005) and confirmed by Mosier and Francis (2008), Auguet et al. (2011) and Lliros et al. (2010) (Figure 3b). Typical freshwater clusters have also been identified within the bacterial domain (Zwart et al 2002). This differentiation of freshwater microbes may be related to physical and chemical properties of the freshwater environment being distinct from terrestrial and marine environments, like nutrient concentrations, mixing and osmotic value (Zwart et al., 2002). Salinity seems to be a selective factor also in lake ecosystems (Scholten et

al., 2005, Hu et al., 2010), but is not likely to play a role in Lake Lucerne, although we observed negative correlation between conductivity and gene-based data in the top layer of this lake (Supplementary Table 1a).

## 5 Thaumarchaeota and environmental factors

Thaumarchaeota are found to thrive in low nutrient environments (e.g. Erguder et al., 2009; Martens-Habbena et al., 2009; Reed et al., 2010), as is characteristic for the oligotrophic lacustrine settings of our study site.

10 The seasonal correlations found between nitrate and archaea in the surface waters of Spanish alpine lakes (Auguet et al., 2011) is confirmed by our study, the latter which additionally showed negative correlations between archaeal numbers and richness on one hand and temperature and conductivity in surface waters on the other hand and indicated that the surface water contained a different seasonality and a less abundant community than the deeper waters.

15 In Lake Lucerne, correlations between abiotic factors and the abundance and richness of the thaumarchaeotal community differed per water depth. Whereas no significant correlation between them was observed at the bottom layer, temperature and conductivity were more important factors in the surface layer, and oxygen and pH in the middle layer at 42 m depth. Temperature and conductivity were also most dynamic in  
20 the surface layer. In soils it was demonstrated that differences in pH could explain the archaeal abundance or species composition (He et al., 2007; Nicol et al., 2008), while oxygen has been shown to be a driving factor in relation to the presence of putative ammonium-oxidizing archaea in the suboxic layer of the Black sea (Coolen et al., 2007, Lam et al., 2007), the Gulf of Mexico (Beman et al., 2008) and even in the oxygen  
25 minimum zone of the ocean, at concentrations of oxygen below the detection level (Lam et al., 2009). As the measured ammonium concentrations were around the detection limit no correlation could be found with the ammonium concentrations in Lake Lucerne.

Archaeal lipids



Simultaneously with the gene-based analyses, specific lipids have been measured in the water column, i.e. crenarchaeol, a membrane lipid specific for thaumarchaeota, which is commonly used as a proxy for thaumarchaeotal biomass (Damste et al., 2002). In Lake Lucerne, however, the expected correlation between crenarchaeol and 16S rRNA gene  
5 copies or cells is not observed throughout the whole water column. As expected the concentration of crenarchaeol in the surface water increases significantly with the 16S rRNA gene copy numbers (Figure 4). However, the amount of crenarchaeol at 42 m water depth does not correlate with the 16S rRNA gene copy or cell numbers. At the deeper waters, the relationship may be lost due to lysis of sinking and decaying cells,  
10 leading to uncoupling between concentrations of lipids and DNA. Another explanation may be the presence of prokaryotes in the deeper waters that are not detected by the primers used, but do contain crenarchaeol. However, the presence of the observed discrepancy between concentrations of crenarchaeol and the 16S rRNA gene throughout all sampling months makes this explanation less likely.

15

#### Conclusions

High numbers of Archaea were found by qPCR in Lake Lucerne compared to Lake Kivu (Lliros et al., 2010) and Lake Pavin (Lehours et al., 2007). They formed a distinct group affiliated with freshwater thaumarchaeota, based on both their functional *amoA* gene  
20 and their 16S rRNA gene and they fall within the *amoA* low saline cluster (Francis et al., 2005; Lliros et al., 2010; Auguet et al., 2011). In freshwater lakes such as Lake Lucerne, these genetically unique thaumarchaeota may play an important role in the nitrogen cycle as has been suggested before for terrestrial and marine ecosystems (Wuchter et al., 2006; He et al., 2007; Beman et al., 2008; Zhang et al., 2010).

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#### **Acknowledgments**

This is publication number DW-2011-1002 of the Darwin Center for Biogeosciences, which partially funded this project. We thank Flavio Anselmetti, Alois Zwysig and Michael Schurter of EAWAG Kastanienbaum for their help during the extensive field

campaigns for which additional financial support was obtained from the Schure-Beijerinck-Popping fund and Darwin Center for Biogeosciences. This is publication number XXXX of the Netherlands Institute of Ecology (NIOO-KNAW).

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## **Titles and legends to figures**

Figure 1: Seasonal distribution of *amoA*- (solid lines) and 16S rRNA- (broken lines) based abundance (circles) and diversity (squares) of thaumarchaea determined at three different layers in the water column of Lake Lucerne. Grey rectangles present the period in which mixing of the water column occurred.

Figure 2: Comparison between archaeal *amoA* and 16S rRNA gene copy numbers determined during a period of 16 consecutive months at three different depths in the water column of Lake Lucerne. Data for the three different water layers have been combined.

Figure 3: Phylogenetic tree of archaeal 16S rRNA (A) and *amoA* protein sequences (B) as retrieved by PCR amplification and gene-cloning of samples of Lake Lucerne in December 2008, which represented all DGGE bands found in this study. For the 16S rRNA gene tree, nodes with a bootstrap value greater than 0.90 or 0.50 are indicated by closed and open circles, respectively. No database sequences were found within the Lake Lucerne clusters.

Figure 4: Amounts of the thaumarchaea-specific lipid crenarchaeol per 16S rRNA gene copy determined at the surface (triangles) and at 42 m depth (circles) of the water column of Lake Lucerne during a sampling period of 16 consecutive months.

## **Title to supplementary table**

Supplementary Table 1: Spearman rank correlation coefficients of biotic and abiotic  
5 factors determined in the water column of Lake Lucerne at three different depths. Shown  
are the values that are statistically significant at  $p < 0.05$ . Gene copy numbers and  
diversity were obtained by qPCR and DGGE band count, respectively.

## Legends to supplementary figures

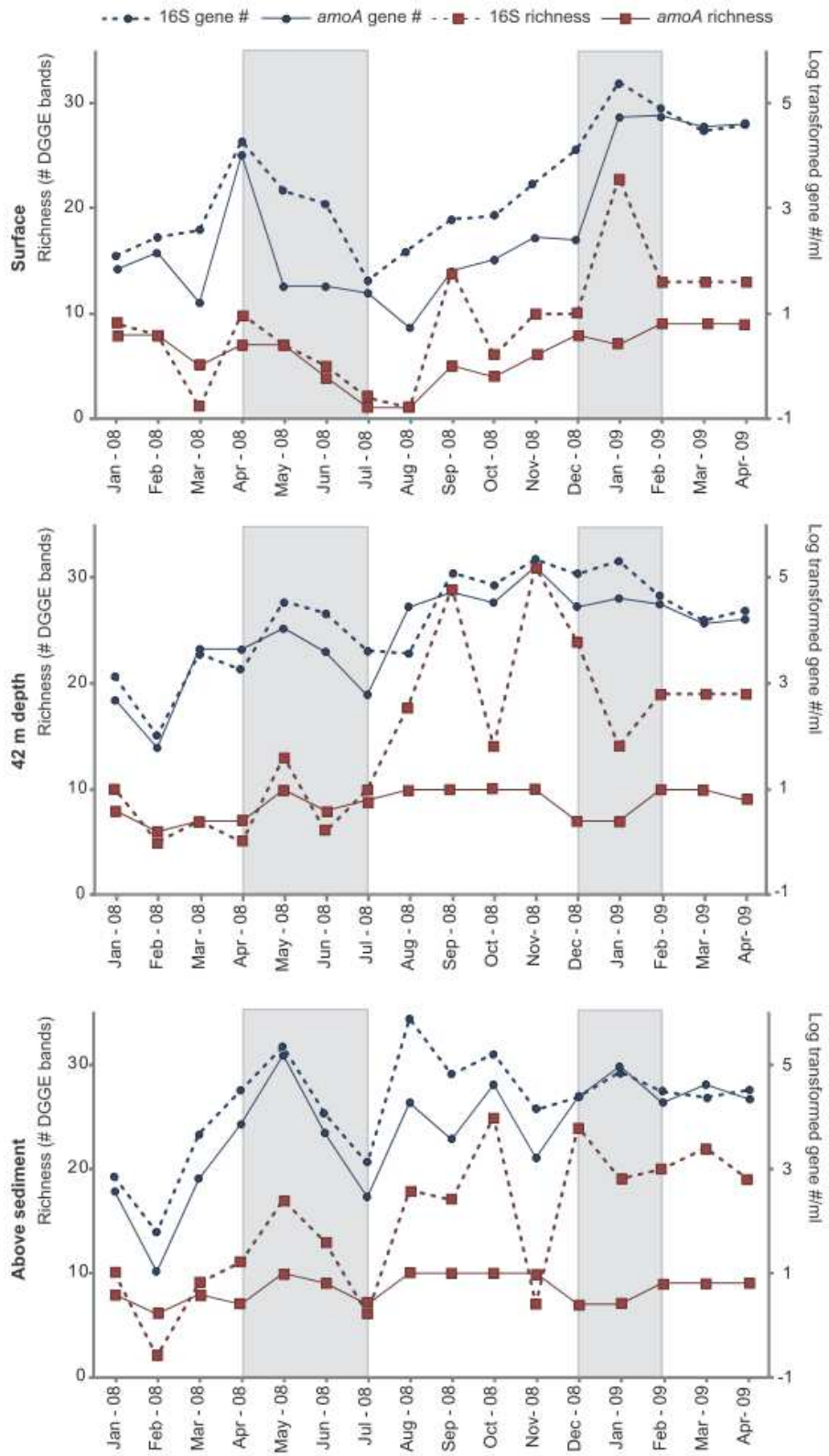
Supplementary Figure 1: Denaturing gradient gel of PCR-amplified 16S rRNA gene fragments obtained from Lake Lucerne in different months and from surface water (top, t), 42 m depth (middle, m) or water above the sediment (bottom, b). The samples of each lane are described by year, month and water depth, respectively, at the top of the figure.

Supplementary Figure 2: Denaturing gradient gel of PCR-amplified *amoA* gene fragments obtained from Lake Lucerne in different months and from surface water (top (t)), 42 m depth (middle (m)) or water above the sediment (bottom (b)). The samples of each lane are described by year, month and water depth, respectively, at the top of the figure.

Supplementary Figure 3: Seasonality of environmental factors and chemical composition at three water depths in Lake Lucerne.



**Figure 1**



**Figure 2**

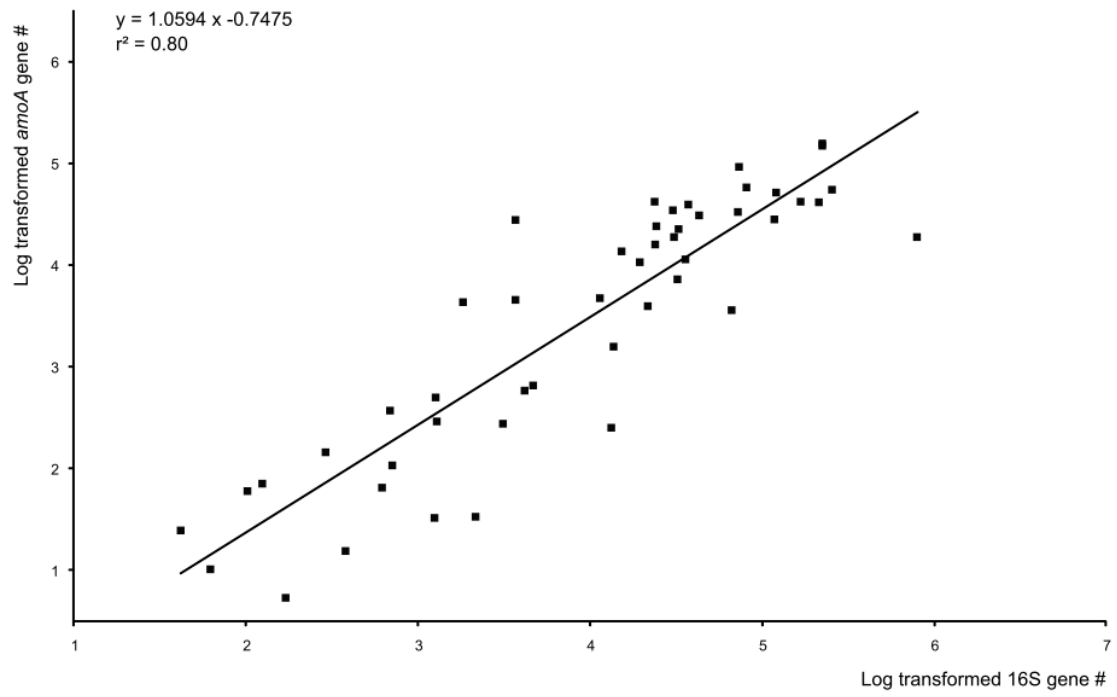
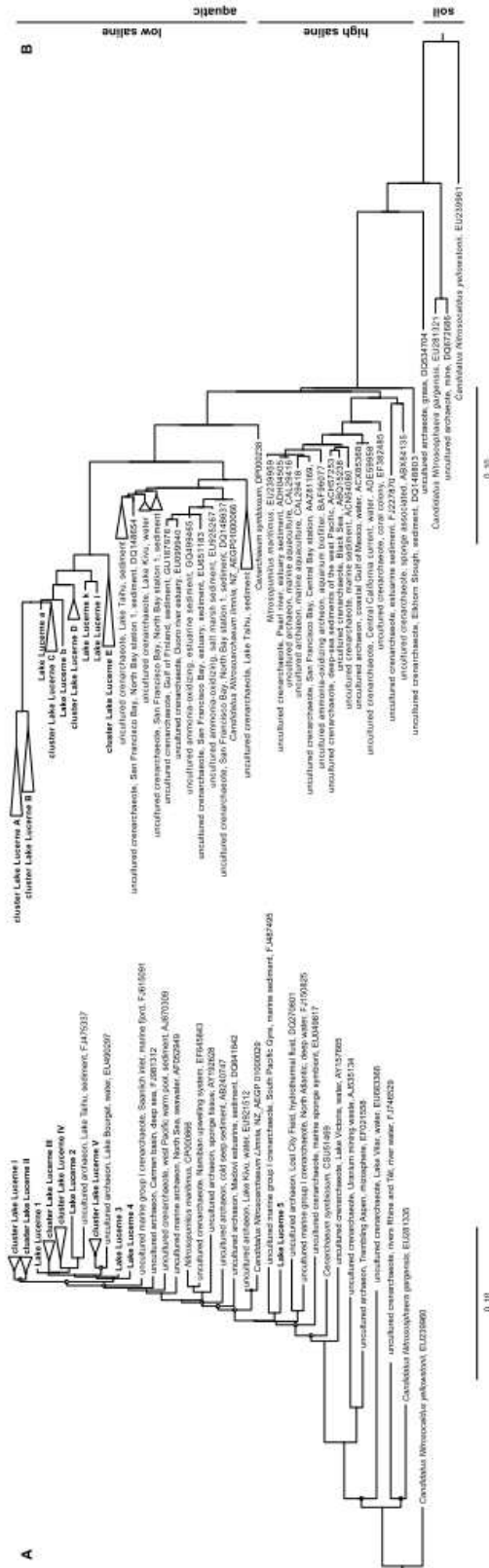
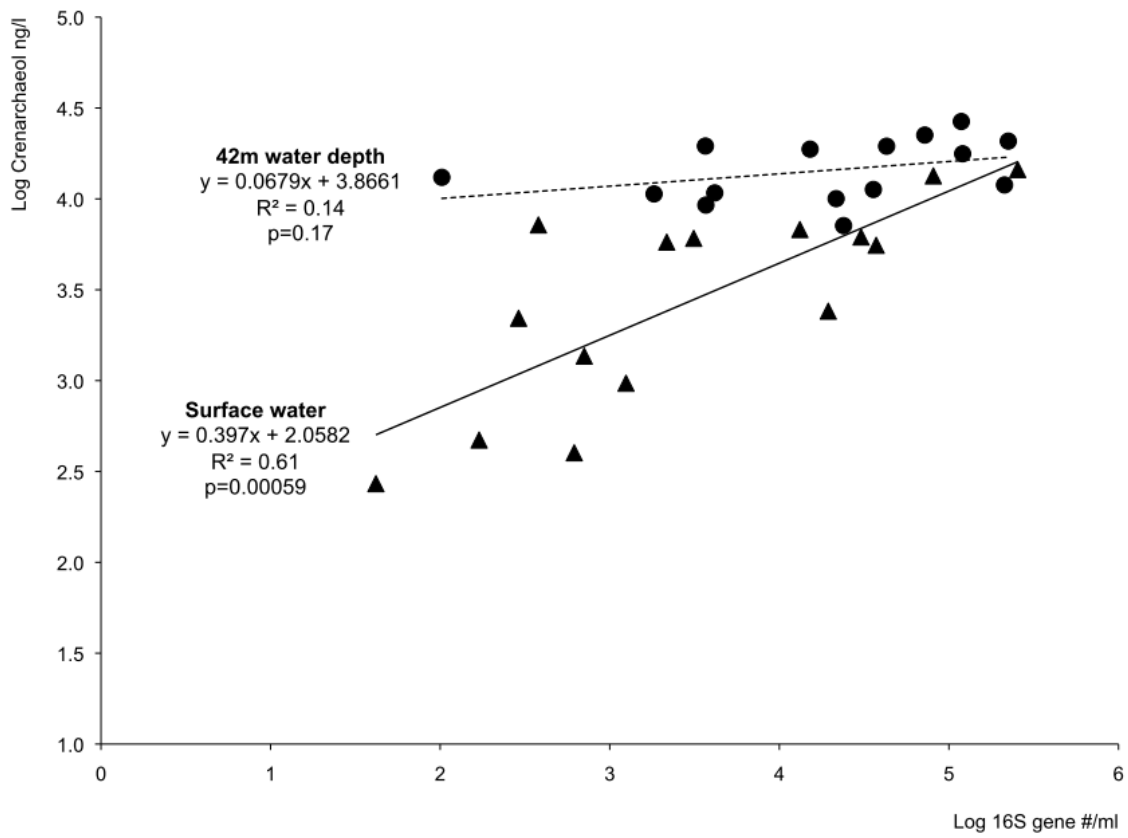


Figure 3



**Figure 4**



## Supplementary Table 1

### A Surface water layer

	16S rRNA gene numbers	amoA gene numbers	16S rRNA gene diversity	amoA gene diversity	NO3	Thaumarchaeol	Temperature	Oxygen	Conductivity	pH
16S rRNA gene numbers	1.000000	0.847059	0.705576	0.594833	0.697802	0.717857	-0.564706			
amoA gene numbers		1.000000	0.834842	0.767334	0.554945	0.603571	-0.700000		-0.564835	
16S rRNA gene diversity			1.000000	0.635218						
amoA gene diversity				1.000000	0.708377	0.642637	-0.856559		-0.767082	
NO3					1.000000		-0.829670		-0.900000	
Thaumarchaeol						1.000000	-0.760714		-0.554945	
Temperature							1.000000		0.903297	
Oxygen								1.000000		
Conductivity									1.000000	
pH										1.000000

### B Water layer of 42 meter depth

	16S rRNA gene numbers	amoA gene numbers	16S rRNA gene diversity	amoA gene diversity	NO3	Thaumarchaeol	Temperature	Oxygen	Conductivity	pH
16S rRNA gene numbers	1.000000	0.876471	0.737630					-0.731868	0.573626	0.652747
amoA gene numbers		1.000000	0.821888	0.511545		0.592857		-0.806593	0.569231	0.727473
16S rRNA gene diversity			1.000000	0.604573	0.712550	0.572719		-0.780991	0.597358	
amoA gene diversity				1.000000						
NO3					1.000000					
Thaumarchaeol						1.000000		-0.681319		0.923077
Temperature							1.000000			
Oxygen								1.000000	-0.740659	-0.775824
Conductivity									1.000000	0.652747
pH										1.000000

### C Water layer 5 meter above the sediment

	16S rRNA gene numbers	amoA gene numbers	16S rRNA gene diversity	amoA gene diversity	NO3	Temperature	Oxygen	Conductivity	pH
16S rRNA gene numbers	1.000000	0.794118	0.631812	0.573439					
amoA gene numbers		1.000000	0.827689						
16S rRNA gene diversity			1.000000						
amoA gene diversity				1.000000					
NO3					1.000000				
Temperature						1.000000		0.846154	
Oxygen							1.000000		
Conductivity								1.000000	
pH									1.000000

**Figure S1**

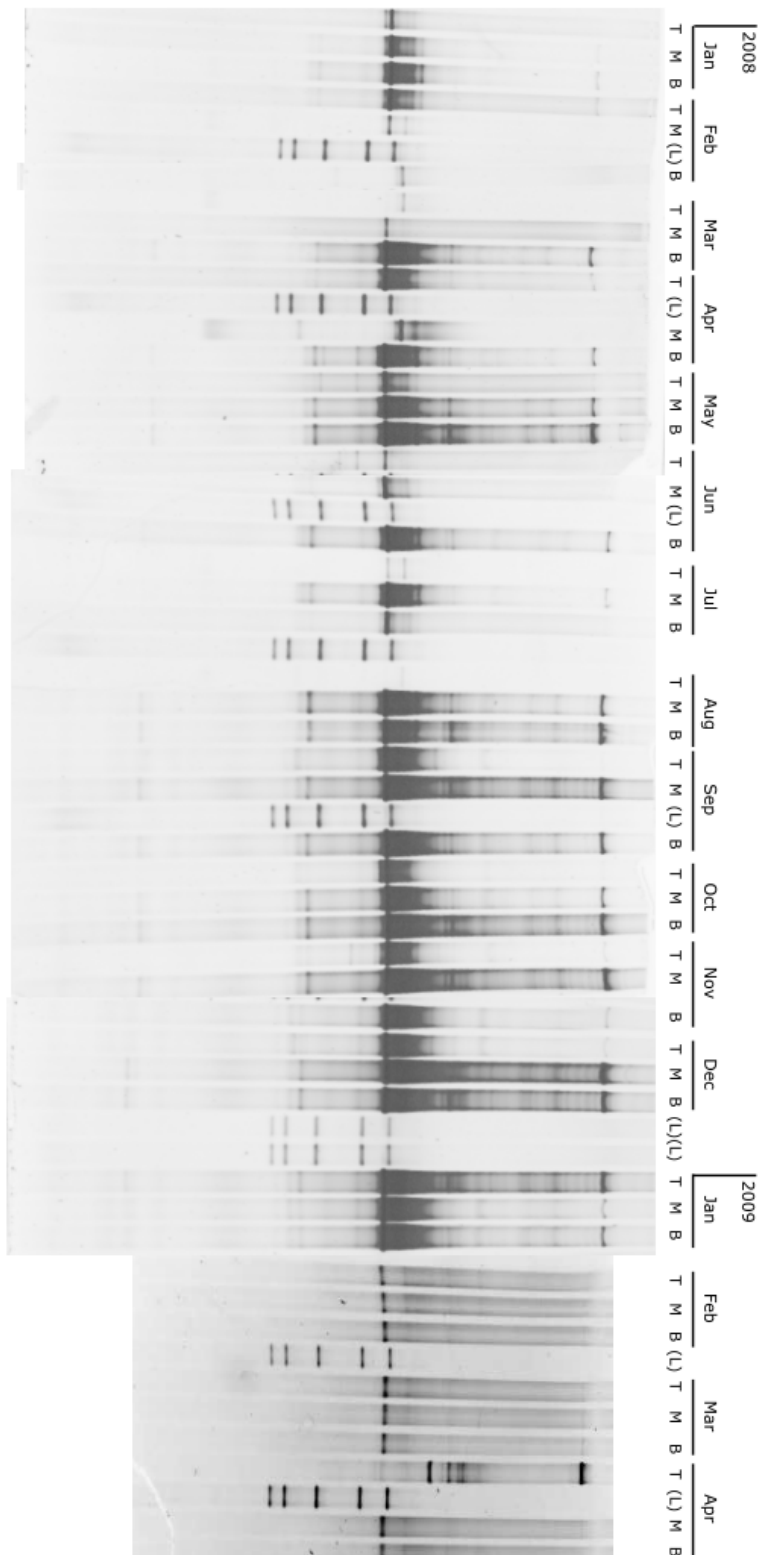
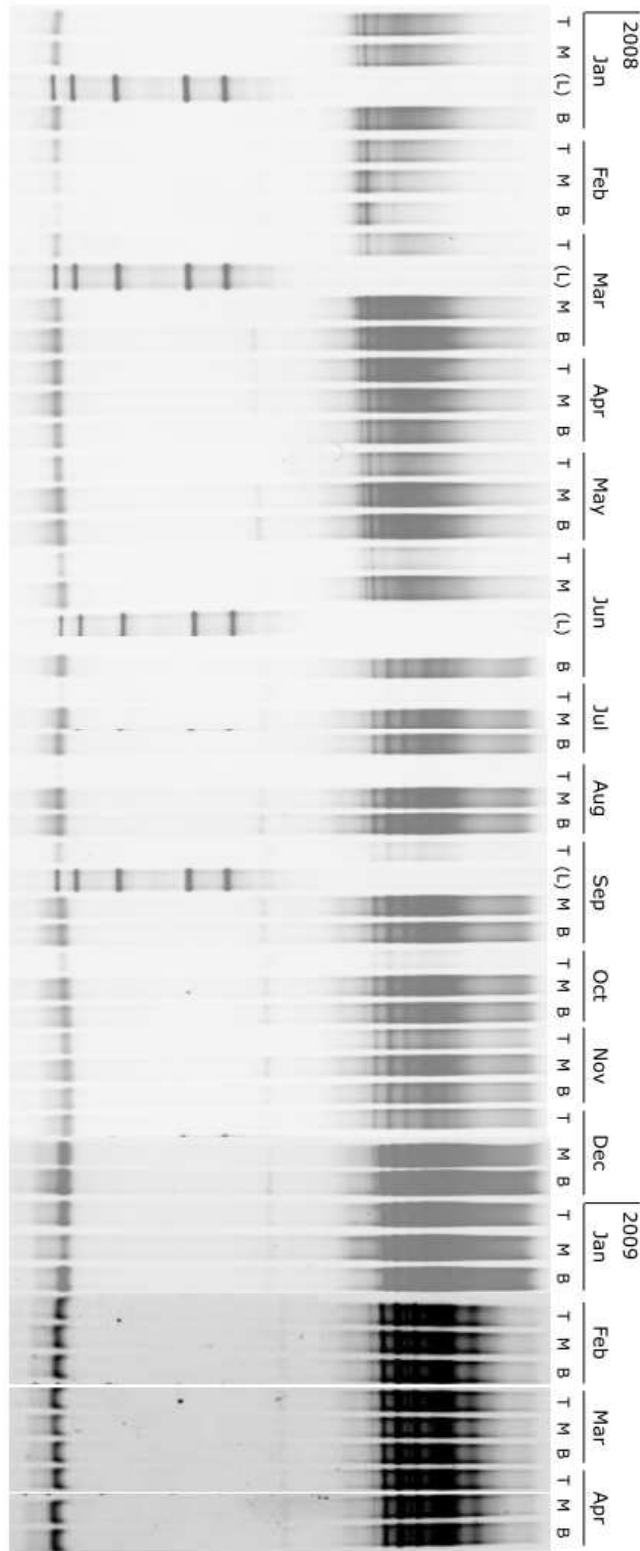


Figure S2



**Figure S3**

