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Ancient European Lakes: Reservoirs of Hidden Microbial Diversity? The Case of Lake Pamvotis (NW Greece)

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Abstract

Ancient European lakes are clustered within a radius of 300 km around Lake Ohrid. Information concerning microbial diversity in these lakes is limited. We studied diversity of the dominant prokaryotic phylotypes in the sediments in one of these lakes, known as Lake Pamvotis. The analysis was performed in samples from two stations for four seasons of the same year. DNA extraction followed by PCR amplification (16S rDNA), Denaturing Gradient Gel Electrophoresis, cloning and sequencing was applied in order to reveal the sequence signatures of the dominant bacterial and archaeal phylotypes. Bacterial and archaeal cell numbers were quantified by real-time PCR. Several environmental variables measured in parallel, including pH, Nickel, Chromium, Arsenic, Calcium, Total Nitrogen and Total Carbon, were found to affect strongly the prokaryotic abundances. Most of the identified sequences of Bacteria belong to *Proteobacteria* and most of the sequences of Archaea belong to *Euryarchaeota*. The great majority of these bacterial (84.21%) and archaeal sequences (95.65%) have no cultivated counterparts in the databases. In addition, many of these bacterial (50.88%) and archaeal sequences (20.65%) correspond to potentially new species. Six of the bacterial sequences constitute a new class of *Cyanobacteria* which we have named "Lake Pamvotis cluster" (LPC). Our findings highlight Lake Pamvotis as a habitat for several previously unidentified species of Bacteria and Archaea.

Keywords

Ancient Lakes, Lake Pamvotis, Bacteria, Archaea

1. Introduction

Worldwide ancient lakes such as Baikal, Tanganyika, Victoria, Titicaca represent "natural laboratories" for evolutionary research and major hotspots of biological diversity [1] [2] [3]. In the European continent, few lakes are old enough to feature endemic species. All of them are restricted to the Balkan Region, a mountainous area in southeastern Europe that has long been recognized as a worldwide hotspot of endemic freshwater biodiversity [4] [5]. The most prominent of these lakes is Lake Ohrid and its sister Lake Prespa with a limnological age of 2 - 5 million years [6] [7]. The majority of all ancient or putatively ancient European lakes are thought to be restricted within a radius of 300 km around Lakes Ohrid and Prespa [5]. This cluster of lakes includes less well known, potentially ancient lakes such as the lakes Skutari (Montenegro, Albania), Mikri Prespa (Greece, Albania), Vegoritis (Greece), Trichonis and the ancient lake Pamvotis (Greece) (**Figure 1**) [2] [8] [9] [10].

Lake Pamvotis has been in existence throughout the Plio-Pleistocene period, as shown by the identification of several endemic mollusc taxa which are known to be 500,000 years old [4]. Therefore, it has attracted research interests as a sedimentary archive on long term environmental and climate history and as a hotspot for European biodiversity. Lake Pamvotis has also been characterized as a Quaternary refugium, that is an ecologically stable area critical not only for the long-term survival of existing species, but also for the emergence of new ones (**Figure 1**) [11].

Unfortunately, microbial diversity has not been extensively studied either in Lake Pamvotis or in other lakes of the wider region. The few studies conducted were mainly focusing on the problems of gradual eutrophication and urbanization in some of these lakes [12] [13] [14] [15]. Nevertheless, the results are

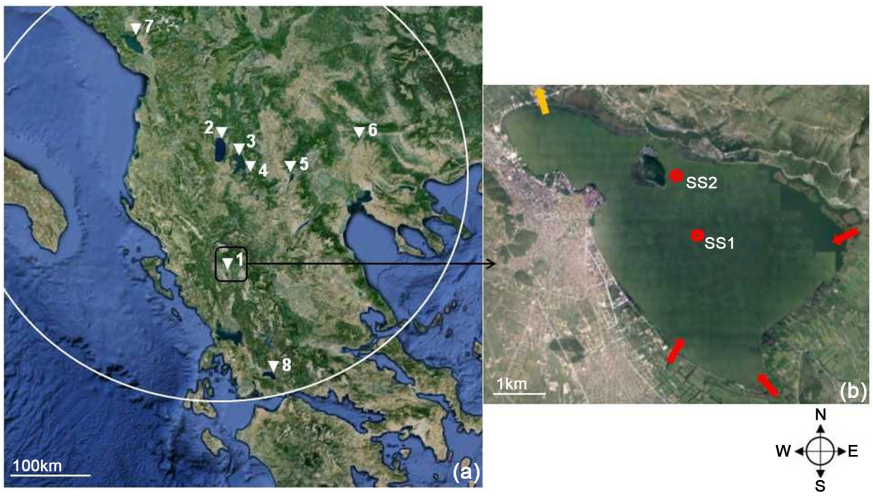


Figure 1. (a) Within a radius of 300 km (white cycle) around Lake Ohrid, are thought to be restricted the most ancient or putatively ancient European lakes [5] (1: Pamvotis, 2: Ohrid, 3: Megali Prespa, 4: Mikri Prespa, 5: Vegoritis, 6: Doirani, 7: Skutari, 8: Trichonis); (b) Sample Stations (SS) in Lake Pamvotis are indicated by dots. Main inflows and outflows are indicated by arrows.

interesting. Molecular data reveal that the population of the filamentous *Cyanobacteria* from Lake Pamvotis is homogeneous, but divergent from other populations worldwide [13]. In the nearby Lake Ziros, all cyanobacterial phylotypes except the ones of three cosmopolitan species (*Planktothrix sp.*, *Anabaena sp.*, *Microcystis sp.*) were found to have low homology to any other known cyanobacterial species [12]. In addition, strains of *Limnothrix redekei* from Lake Kastoria, a potentially ancient lake in the same region, form a separate phylogenetic group within the *Cyanobacteria* [16]. Novel phylotypes belonging to the *Chroococcales* were recognized recently in lakes Kastoria and Doirani [14]. Bacterial diversity in the water and sediment of lake Kastoria was found to be high, consisting mostly of yet uncultured Bacteria, whereas 11% of the water column and 5% of the sediment bacterial phylotypes could not be classified with any of the known bacterial phyla [15]. The results from those studies indicate the existence of a significant hidden microbial diversity in these ancient ecosystems. However, a systematic study of the bacterial diversity has not been undertaken to date in any of these lakes and; in addition, the abundance and diversity of Archaea has not been investigated at all. In this study, we present a systematic analysis of both the bacterial and the archaeal dominant phylotypes in the sediments of Lake Pamvotis.

Our study addresses three important questions on the organization of this aquatic microbial ecosystem: 1) Are there novel, previously unidentified, bacterial and archaeal species among the dominant phylotypes? 2) Are archaeal communities a quantitatively important component of microbial communities inhabiting this environment? 3) Is there a correlation between physicochemical variables, prokaryotic abundance and diversity of the dominant phylotypes?

2. Materials and Methods

2.1. Sampling Sites and Sample Collection

Lake Pamvotis is a closed hydrological system. It lies approximately at 39˚40'N, 20˚53'E, at 470 meters above sea-level in the mountainous region of the Pindus. It is a shallow lake (4.23 m average depth) and has a surface area of about 22.8 km^2 [13].

Sediment samples (top 5 - 10 cm) were collected using a grab sampler at two sampling stations (SS): SS1 and SS2. SS1 is situated approximately in the middle of the lake (depth 6.5 to 7.5 m depending on the season) and SS2 is a station where the maximum depth of the lake was measured (8.5 to 9.5 m depending on the season) (**Figure 1**). Temperature was measured in water just above the sediment by a depth sampling device with a built-in thermometer (Windaus, Labortechnic, GmbH 7 Co.KG). By using a GPS instrument, we collected samples from the same sites once per season over a one-year period (the year 2012). Once retrieved onboard, sediments were homogenized and sub-sampled in sterilized Falcon tubes for DNA extraction and for physicochemical analysis. Subsamples were transported to the laboratory in a portable freezer in less than an hour.

2.2. Chemical Analysis of Sediment Samples

Sediment samples were dried at 70˚C for 24 h upon arrival to the laboratory.

For the pH measurements, sediment samples were diluted in 1M KCl (1:2 sediment to solution ratio) and a Hanna pH meter was used (Hanna Instruments pH211) [17].

Two grams of each sample were extracted twice with 20 mL of bidistilled water, for anions (Cl^-, SO_4^{2-}) analysis, and 20 mL of 40 mM nitric acid aqueous solution, for cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) analysis, in an ultrasonic bath for 30 min. The extracts were centrifuged, combined and diluted in bidistilled water to a volume of 50 mL. 20 µL of each sample were injected in HPLC equipped with a conductivity detector (Shimadzu CDD-10A VP). For the determination of cations IC YK-421 column with a Shodex IC YK-G column guard and anions IC NI-424 column with IC NI-G column guard in a Shimadzu CTO-10AC column oven were used with shipping solvent. Standard solutions of the above ions at concentrations ranging from 1 to 100 mg/L in seven levels were analyzed as external calibration basis quantification [18].

Total carbon (TC) and total organic carbon (TOC) were analyzed with a Shimadzu TOC-VCPH carbon analyzer (Shimadzu, Japan), coupled to a solid state combustion unit (model SSM-5000A). One gram of dried sample was inserted in solid state combustion unit. For TC the unit uses catalytically aided combustion oxidation at 900˚C method and for inorganic carbon (IC) pre-acidification, with oven temperature 250˚C. After the treatment in the solid state combustion unit, samples were automatically inserted directly in the carbon analyzer, which measures the TC and IC. TOC was derived by subtracting the IC from the TC.

The total nitrogen in the sediment (TN) was determined spectrophotometrically by Total Kjeldahl (Nessler method) after digestion by the HACH Digesdahl Apparatus together with 3 mL H_2SO_4 (98% v/v) at 450˚C, while for the amendment of the digest the HACH method 8075 was used. The concentration of TN within the sample was measured in a HACH DR/2010 Spectrophotometer at the wavelength of 460 nm. The total phosphorous content (TP) in the sediment was determined by the molybdenum blue method (HACH) [19].

Heavy metals Sb, Ni, Hg, Se, Cd, Mn, Pb, Fe, Cu, Cr, Zn and As were determined using ICP-AES (Thermo Scientific iCAP 6300 ICP Spectrometer) according to the methodology described by Ashley *et al.* [20].

2.3. Isolation of Culturable Bacteria

For the isolation of culturable bacterial species, R2A plates (LABM, United Kingdom) were prepared according to the manufacturer's instructions. R2A medium was used for a general view of culturable freshwater Bacteria. Ten grams of sediment samples taken during summer from both stations were suspended in sterile water. A series of 10-fold dilutions were prepared. R2A medium plates were inoculated with 100 µL aliquots from different dilutions as described earlier [21]. Plates were incubated at 26˚C (since bottom water tempera-

ture during summer ranged from 24˚C to 26˚C, Table 1) for 10 days in the dark. Bacterial colonies were selected based on morphological features and color [22].

2.4. DNA Extraction, PCR Amplification and Quantitative Real-Time PCR

DNA was extracted from the sediment samples using an UltraClean soil DNA isolation kit from MoBio Laboratories (PowerSoil DNA Isolation kit, Carlsbad, CA 92010) in accordance with the manufacturer's instructions.

PCR amplification was performed in a Biorad iCycler in a 50 µL reaction volume. For archaeal 16S rDNA amplification, a 344F-GC and 915R primer set was used and a touchdown PCR was performed as described earlier [23].

For bacterial 16S rDNA amplification a 341F-GC and 907R primer set was used and a touchdown PCR was performed as described earlier [24].

PCR products for both Archaea and Bacteria 16S rDNA were evaluated in a 1% (w/v) agarose gel electrophoresis and subsequently used for Denaturing Gradient Gel Electrophoresis (DGGE).

For quantification of archaeal and bacterial 16S rRNA genes in our samples, serial 10-fold dilutions of recombinant plasmids containing a partial fragment of an archaeal and a bacterial 16S rDNA respectively were used as external standards, to obtain a reference curve. The standard dilutions ranged from 10^3 to 10^5 and from 10^4 to 10^{10} for archaeal and bacterial reference curves, respectively.

The real-time PCR was performed in a LightCycler 480 (Roche) instrument using the LightCycler 480 SYBR Green Master I (Roche) following the manufacturer's instructions. The final 20 µL reaction mix contained 10 µL of the SYBR Green Master Mix I, the original primer set (in case of Forward primers without the GC clamp) for Bacteria and Archaea and an appropriate dilution of the DNA samples were initially incubated at 95˚C for 5 min followed by 40 cycles of a 3-step cycling at 95˚C for 45 s (denaturation), 61˚C for 45 s for Archaea or 60˚C for 45 s for Bacteria (annealing), 72˚C for 45 s (extension) and a final extension for 10 min at 72˚C. All samples, standards and negative controls were tested in triplicates. Finally, we used CT values to determine the 16S rDNA copy numbers in our samples and we converted them into cell numbers assuming that archaeal cells contain 2 and bacterial cells contain 3.8 16S rDNA copies per cell [25].

2.5. Denaturing Gradient Gel Electrophoresis (DGGE), Cloning and Sequencing

DGGE for Archaea and Bacteria was performed as described earlier by Muyzer *et al*. [26] with minor modifications as described by Janse *et al*. [13] [27]. We used a denaturing gradient 20% - 70% and 20% - 60% for Archaea and Bacteria respectively. Bands were detected after ethidium bromide staining, excised and incubated in 50 µL sterile MilliQ water O/N at 4˚C. A new PCR was performed using the eluent and the original primer set and run on a DGGE gel to confirm its identity. The PCR products were purified using a Macherey-Nagel DNA clean-up kit (NucleoSpin Gel and PCR Clean-up, Duren-Germany), and

Table 1. Physical-chemical properties of Lake Pamvotis sediments.

	Depth (m)	T˚C	pH	TC (mg/g)	TOC (mg/g)	TP (mg/g)	TN (mg/g)
Spring, SS1	8.40 ± 0.20	20 ± 0.50	6.96 ± 0.02	67.54 ± 7.26	60.69 ± 3.13	3.02 ± 0.02	2.94 ± 0.01
Spring, SS2	9.30 ± 0.30	19 ± 0.40	6.30 ± 0.01	99.79 ± 3.53	87.31 ± 3.99	4.05 ± 0.02	5.03 ± 0.11
Summer, SS1	7.30 ± 0.15	26 ± 0.70	7.07 ± 0.01	68.58 ± 2.31	65.00 ± 2.01	4.83 ± 0.05	3.91 ± 0.03
Summer, SS2	8.20 ± 0.18	24 ± 0.50	6.31 ± 0.01	99.53 ± 2.07	93.70 ± 4.09	4.13 ± 0.02	3.33 ± 0.02
Autumn, SS1	8.10 ± 0.25	11 ± 0.50	7.08 ± 0.01	67.39 ± 2.42	63.54 ± 2.56	10.01 ± 0.09	3.99 ± 0.03
Autumn, SS2	9.00 ± 0.20	12 ± 0.50	6.23 ± 0.02	65.60 ± 1.01	61.30 ± 1.96	9.69 ± 0.08	5.01 ± 0.05
Winter, SS1	8.40 ± 0.10	6 ± 0.10	7.18 ± 0.20	59.73 ± 3.60	58.19 ± 2.12	10.75 ± 0.42	3.94 ± 0.03
Winter, SS2	9.30 ± 0.40	6 ± 0.20	6.45 ± 0.02	79.80 ± 11.14	79.60 ± 1.02	8.78 ± 0.05	5.80 ± 0.01

(a)

	Na^+ (mg/kg)	K^+ (mg/kg)	Ca^{2+} (mg/kg)	Mg^{2+} (mg/kg)	Cl^- (mg/kg)	SO_4^{2-} (mg/kg)
Spring, SS1	4.96 ± 0.01	4.17 ± 0.01	37.69 ± 0.02	13.06 ± 0.04	64.34 ± 0.02	296.62 ± 0.04
Spring, SS2	6.09 ± 0.01	5.41 ± 0.01	72.15 ± 0.26	17.83 ± 0.01	117.82 ± 0.70	537.42 ± 0.10
Summer, SS1	4.87 ± 0.01	4.34 ± 0.01	50.04 ± 0.02	14.48 ± 0.03	64.66 ± 0.02	554.17 ± 0.03
Summer, SS2	5.54 ± 0.01	5.98 ± 0.01	76.34 ± 0.79	19.41 ± 0.04	103.95 ± 0.18	386.02 ± 0.12
Autumn, SS1	4.91 ± 0.01	5.57 ± 0.03	49.19 ± 0.12	18.73 ± 0.02	70.17 ± 0.01	431.68 ± 0.02
Autumn, SS2	6.33 ± 0.01	5.86 ± 0.01	70.98 ± 0.02	18.95 ± 0.03	109.87 ± 0.09	589.16 ± 0.03
Winter, SS1	3.39 ± 0.01	5.56 ± 0.01	57.28 ± 0.02	16.33 ± 0.02	35.35 ± 0.02	450.01 ± 0.03
Winter, SS2	3.62 ± 0.02	5.02 ± 0.02	51.26 ± 0.73	16.32 ± 0.02	51.16 ± 0.04	428.55 ± 0.07

(b)

	Sb (mg/kg)	Ni (mg/kg)	Hg (mg/kg)	Se (mg/kg)	Cd (mg/kg)	Mn (mg/kg)
Spring, SS1	5.10 ± 0.08	**132.00 ± 0.25**	**1.13 ± 0.01**	<6.00	<4.00	1090.00 ± 22.00
Spring, SS2	2.43 ± 0.02	**98.00 ± 1.75**	**1.59 ± 0.01**	<6.00	<4.00	959.00 ± 10.00
Summer, SS1	2.85 ± 0.02	**126.00 ± 0.20**	0.16 ± 0.01	<6.00	<4.00	1130.00 ± 35.00
Summer, SS2	2.78 ± 0.01	**97.10 ± 1.53**	**0.84 ± 0.02**	<6.00	<4.00	843.00 ± 21.00
Autumn, SS1	2.89 ± 0.01	**123.00 ± 0.17**	**0.44 ± 0.01**	<6.00	<4.00	1330.00 ± 76.00
Autumn, SS2	2.72 ± 0.02	**96.50 ± 1.20**	**0.95 ± 0.01**	<6.00	<4.00	923.00 ± 15.00
Winter, SS1	3.32 ± 0.02	**135.00 ± 0.22**	0.18 ± 0.01	<6.00	<4.00	950.00 ± 15.00
Winter, SS2	3.52 ± 0.02	**88.50 ± 2.70**	<0.10	<6.00	<4.00	999.00 ± 32.00

(c)

	Pb (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Cr (mg/kg)	Zn (mg/kg)	As (mg/kg)
Spring, SS1	<30.0	25200.00 ± 58.00	31.40 ± 1.10	**83.10 ± 2.87**	81.30 ± 1.99	2.76 ± 0.01
Spring, SS2	<30.0	25500.00 ± 61.00	**31.80 ± 0.90**	**59.40 ± 2.12**	89.90 ± 3.22	4.58 ± 0.02
Summer, SS1	<30.0	27600.00 ± 32.00	**32.10 ± 0.70**	**83.60 ± 2.66**	93.00 ± 1.42	1.88 ± 0.01
Summer, SS2	<30.0	26400.00 ± 59.00	**32.10 ± 1.20**	**66.00 ± 1.89**	91.50 ± 2.89	4.44 ± 0.03
Autumn, SS1	<30.0	28100.00 ± 45.00	30.60 ± 0.50	**81.70 ± 1.57**	90.10 ± 1.08	2.40 ± 0.02
Autumn, SS2	<30.0	23600.00 ± 52.00	31.00 ± 0.20	**63.60 ± 1.75**	97.00 ± 3.55	4.37 ± 0.01
Winter, SS1	<30.0	28200.00 ± 42.00	**34.00 ± 0.90**	**79.20 ± 1.28**	97.00 ± 2.80	2.14 ± 0.01
Winter, SS2	<30.0	21000.00 ± 20.00	**37.00 ± 0.40**	**58.40 ± 1.97**	78.70 ± 1.43	4.80 ± 0.02

Physicochemical properties of the sediments in Lake Pamvotis sample station 1 (SS1) and 2 (SS2). (a) Depth, T, pH, Carbon, Nitrogen and Phosphorous contents; (b) Major anions and cations; c) Heavy metals. Heavy metal concentrations exceeding the PEC or TEC limits are indicated in bold (Ni PEC: 48.6 mg/kg, Hg PEC: 1.06 mg/kg, Hg TEC: 0.18 mg/kg, Cr TEC: 43.4 mg/kg, Cu TEC: 31.6 mg/kg) [36].

afterwards they were cloned using a TOPO TA cloning Kit (Invitrogen, USA) according to the manufacturer's instructions. Subsequently, ten recombinant clones from each library (corresponding to each DGGE band) were randomly picked for further analysis. Inserts were digested with restriction enzyme HaeIII (HT Biotechnology Ltd, Cambridge, United Kingdom) in order to identify different Restriction Fragment Length Polymorphisms (RFLPs) [28]. Clones with different restriction patterns were sequenced at both strands. Sequencing was performed by Eurofins Genomics/VBC Biotech (Austria) [13] [28].

2.6. Nucleotide Sequences and Accession Numbers

The final sequences were deposited at GenBank and were assigned accession numbers KC510289-KC510380 for Archaea, KP244158-KP244214 for Bacteria and KU862661-KU862683 for cultured isolates.

2.7. Phylogenetic Trees and Statistical Analysis

All sequences were compared against GenBank using BLAST in order to obtain their phylogenetic affiliation. Phylogenetic analyses were performed with MEGA6.1 software. Trees were constructed using the Neighbor-Joining method with Jukes-Cantor distance correction [29].

Spearman's correlation coefficient was used to investigate possible relationships among bacterial and archaeal abundances and the physicochemical variables. All statistical analyses were conducted with STATISTICA 7 (Tulsa, OK, USA).

3. Results and Discussion

3.1. Physical-Chemical Properties of Lake Pamvotis Sediments

Total Carbon (TC), Total Organic Carbon (TOC), Total Nitrogen (TN) and Total Phosphorus (TP) concentrations (**Table 1**) are in accordance to previously published studies underlining the eutrophic status of the lake [19] [30] [31]. Moreover, TN, TP and TOC concentrations in Lake Pamvotis sediments are comparable to those measured in other lakes worldwide [32] [33] [34] [35].

Concerning heavy metal concentrations, according to the Sediment Quality Guidelines (SQGs) [36], only Ni concentrations exceeded the Probable Effect Concentration (PEC) in Lake Pamvotis sediments in both stations during all seasons. Mercury concentrations exceeded PEC only at spring. Two other heavy metals (Cr and Cu) were found to exceed the Threshold Effect Concentration (TEC) (**Table 1**).

In a previous study conducted between 1991-1993 heavy metal concentrations had been measured in surface sediment samples from Lake Pamvotis stations SS1 and SS2 [37]. It appears that the average Ni concentration in the lake has been increased between 1991 [37] and 2012 (our current study). More specifically, the Ni concentration is 4.7- to 5.1-fold higher than 1991-1993 in SS1 and 1.8- to 2.0-fold higher in SS2.

Nickel and Cr input in lake sediments are possibly enhanced either by mining activities [38] or by incompletely treated industrial and municipal wastewaters, agrochemicals, landfill leachates [39]. In the case of Lake Pamvotis, a municipal wastewater treatment plant exists since 1992, the industrial and agricultural activities have declined since 1990 and there are no mining activities. Thus, the most reasonable explanation for the elevated amounts of Ni at present times is the accumulation of geogenic material draining from the SE due to the construction of a four-km long tunnel at the Mitsikeli Mountain in years 1999-2007.

Mercury (Hg) concentrations in Lake Pamvotis sediments remains stable relative to the concentrations measured previously (1991-1993) [37]. Concerning the presence of As in both stations we cannot speculate on the origins, due to the lack of previous studies.

In a recent study [40], Lake Pamvotis sediments have been characterized as moderately to severely contaminated with heavy metals. Municipal wastewater, silver smithy and operation of leather tanneries from the 17th until the mid-20th century are assumed to be the main reasons for metal contamination [40].

3.2. Prokaryotic Abundance in Lake Pamvotis Sediments: Bacteria vs Archaea

The prokaryotic community in the Lake Pamvotis sediments was found to be dominated by Bacteria. Archaea accounted for 6.17% to 14.09% of the total prokaryotic 16S rDNA copy number (Table 2). Taking into account the average 16S rDNA copy number in archaeal (2 copies/cell) and bacterial (3.8 copies/cell) genomes [25], we can estimate that Archaea may represent 11.13% to 23.88% of the total prokaryotic cells in Lake Pamvotis (Table 2).

Our data are in agreement with previously published studies on other lakes suggesting that Archaea are not the dominant component of the prokaryotic community in freshwater sediments. In sediments of Lake Pavin, qPCR analysis

Table 2. Quantification of bacterial and archaeal cell numbers in Lake Pamvotis sediments.

	Bacterial 16S rDNA copies/g sediment	Archaeal 16S rDNA copies/g sediment	%Archaeal 16S rDNA copies	Bacteria estimated cell number/g sediment	Archaea estimated cell number/g sediment	%Archaea cell number
Spring, SS1	$3.16 \pm 0.29 \times 10^9$	$2.08 \pm 0.21 \times 10^8$	6.17%	0.83×10^9	1.04×10^8	11.13%
Summer, SS1	$4.52 \pm 0.24 \times 10^9$	$5.24 \pm 0.18 \times 10^8$	10.38%	1.18×10^9	2.62×10^8	18.16%
Autumn, SS1	$4.04 \pm 0.22 \times 10^9$	$3.94 \pm 0.23 \times 10^8$	8.88%	1.06×10^9	1.97×10^8	15.30%
Winter, SS1	$4.76 \pm 0.26 \times 10^9$	$3.25 \pm 0.20 \times 10^8$	6.39%	1.25×10^9	1.62×10^8	11.47%
Spring, SS2	$4.32 \pm 0.21 \times 10^9$	$6.32 \pm 0.19 \times 10^8$	12.76%	1.13×10^9	3.16×10^8	21.85%
Summer, SS2	$5.24 \pm 0.23 \times 10^9$	$8.60 \pm 0.24 \times 10^8$	14.09%	1.37×10^9	4.30×10^8	23.88%
Autumn, SS2	$5.68 \pm 0.28 \times 10^9$	$8.12 \pm 0.26 \times 10^8$	12.54%	1.49×10^9	4.06×10^8	21.41%
Winter, SS2	$5.64 \pm 0.27 \times 10^9$	$7.68 \pm 0.22 \times 10^8$	11.98%	1.48×10^9	3.84×10^8	20.96%

Quantification of both bacterial and archaeal 16S rDNA gene copies in Lake Pamvotis sediments, as determined by quantitative PCR assays. Bacterial and archaeal cell numbers have been estimated assuming 3.8 and 2 copies of the 16S rDNA per bacterial and archaeal cell, respectively [25].

revealed that Archaea accounted for 5% - 18% of the prokaryotic community [35]. Furthermore, in sediments of Lake Taihu the archaeal 16S rDNA in the total prokaryotic community ranged from 14.7% to 96.9% [41]. Generally, Archaea are dominant mainly in prokaryotic communities of the deep marine subsurface and saline lake sediments [35] [41] [42] [43] [44].

Based on our results, SS2 displays higher abundances for both bacterial and archaeal communities. Spring is the period of the year where both bacterial and archaeal numbers are lower, whereas the highest abundances are recorded in summer (**Table 2**).

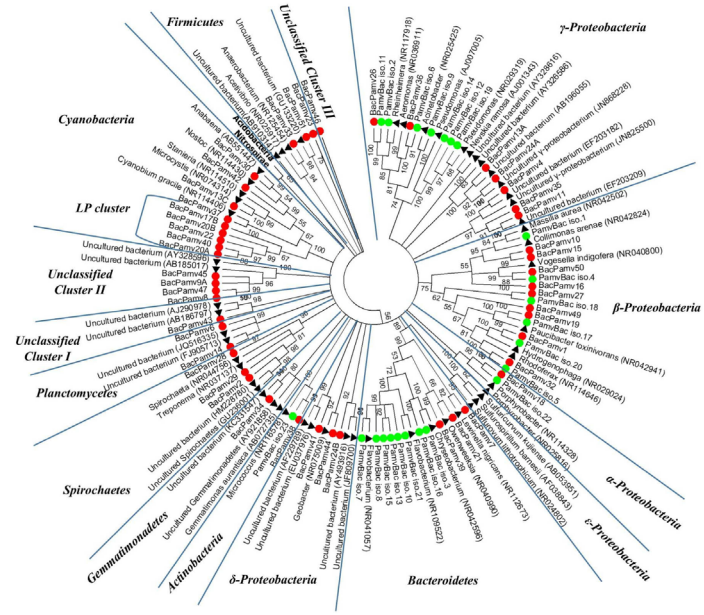
3.3. Diversity of the Dominant Bacterial Phylotypes in Lake Pamvotis Sediments

A total of 153 DGGE bands were identified (**Figure S1**), processed as described in Methods and found to correspond to 57 unique sequences, most of which are novel. Twenty-nine of these sequences (50.88%), were found to have <97% identity to already deposited Genbank entries. Moreover, 48 of these sequences (84.21%) were found to have <97% identity to already known cultivated bacterial species (**Table S1**).

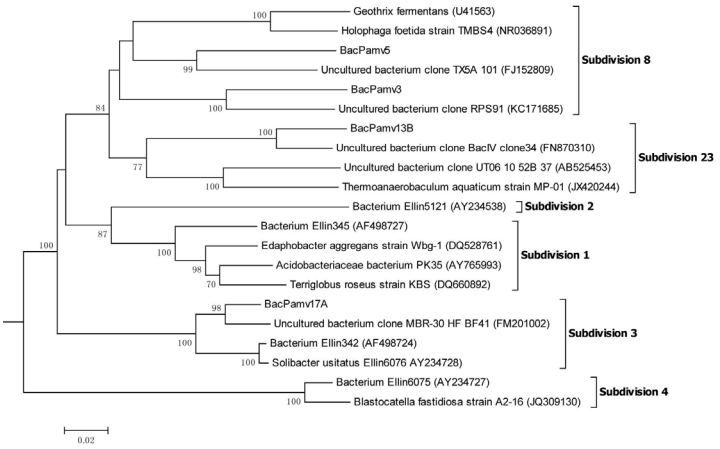
Is this bacterial diversity recognizable also with common cultivating techniques? To address this question, R2A plates were inoculated as described in Methods. A total of fifty randomly selected bacterial colonies were grown and characterized further. Of these 50 colonies, 23 different bacterial phylotypes were identified based on 16S rDNA sequences. Interestingly, 13.04% of these sequences, were found to have <97% identity to already deposited Genbank entries (**Table S2**).

Based on the constructed phylogenetic tree (**Figures 2(a)-(c)**), the DGGE-retrieved sequences (BacPamv; red symbols in **Figure 2(a)**) revealed that the bacterial community of the sediments in Lake Pamvotis comprised mainly of *Proteobacteria* (*β*-, *γ*-, *δ*- and *α*-*Proteobacteria*), followed by phylotypes belonging to *Cyanobacteria*, *Nitrospirae*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, *Spirochaetes*, *Planctomycetes*, *Actinobacteria*, *Gemmatimonadetes*. We also found six sequences which were not affiliated to any known class and were designated as "unclassified" Bacteria (Unclassified Clusters I, II, and III; **Figure 2(a)**).

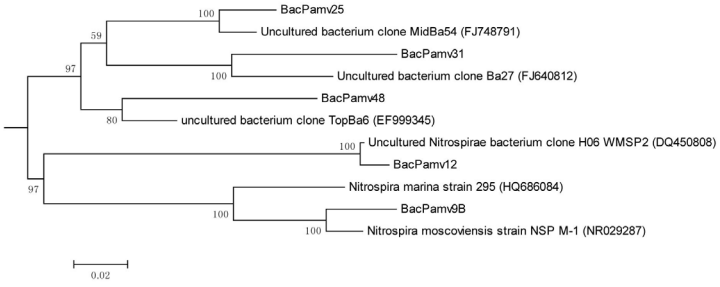
More specifically, most of the DGGE-retrieved Proteobacterial sequences are contained in the class *β*-*Proteobacteria* (9 sequences). Four of them have low identity to any known bacterial sequences (<94%) (**Figure 2(a)**). This group of Bacteria is often the most abundant in freshwater lakes [45] [46] [47] [48]. In our study, members of *β*-*Proteobacteria* were identified in both stations and during all seasons (**Table S3**). Concerning the 23 Bacteria isolated in culture from Lake Pamvotis sediments (PamvBac iso; green symbols in **Figure 2(a)**) six of them were found to be *β*-*Proteobacteria*. Interestingly one 16S rDNA sequence corresponding to the cultivated bacterium PamvBac iso.18, displays < 93% identity to already known 16S rDNA sequences (**Table S2**).



(a)



(b)



(c)

Figure 2. (a) Distance tree based on the alignment of bacterial 16S rDNA sequences from Lake Pamvotis sediments (● BacPamv, ● PamvBac iso) and (▲) a number of sequences with the highest similarities retrieved from GenBank/EMBL/DDBJ databases (Branches with bootstrap values below 50% have been deleted in this presentation); (b) Phylogenetic tree of *Acidobacteria*-like 16S rDNA sequences (Bootstrap values are shown next to the branches); (c) Phylogenetic tree of *Nitrospirae*-like 16S rDNA sequences (Bootstrap values are shown next to the branches).

Cyanobacterial clones were identified in both stations and during all seasons (**Table S3**). A cyanobacterial clone was strongly related to *Microcystis sp*. (99%), a second one to *Cyanobium sp.* (99%) and two other sequences were related to *Nostocacceae Cyanobacteria* although with low identities (92% - 95%). The remaining five cyanobacterial clones displayed strikingly low identity percentages (79% - 85%) compared to any other already identified sequence. These "low-identity" sequences might represent either benthic *Cyanobacteria* or hibernating forms of planktonic *Cyanobacteria*. It has been shown that lake sediments serve as a storage depot (reservoir) for cyanobacterial cells [49].

In lake Pamvotis, two distinct planktonic cyanobacterial populations had been identified previously, based on internal transcribed spacer (ITS) analysis. One of them was defined as *Microcystis sp.* and the other one consisted of various filamentous *Cyanobacteria* which comprise a phylogenetically diverse group unprecedented by other populations worldwide [13]. Based also on ITS data, *Cyanobacteria* species/strains in two other lakes of the wider area were found to have low identities to other known ITS sequences with the exception of some well characterized cosmopolitan species [12] [50]. These observations led to the notion that the presently unknown species/strains might be endemic in these lakes [50]. It has recently been proposed that in the case of algae (including phytoplankton), the "everything is everywhere" hypothesis should be abandoned since algae are neither cosmopolitan nor ubiquitous [51] [52]. Given that homologies between 16S rDNA sequences are higher than those between ITS, the identification of cyanobacterial 16S rDNA sequences with very low homologies to other existing sequences worldwide strengthens the notion that putatively endemic species are present in Lake Pamvotis. Moreover, the relevant "low-identity" sequences (BacPamv 17B, 20A, 20B, 22, 40) form a robust cluster in the constructed phylogenetic tree (**Figure 2(a)**), which was designated "LP cluster" (LPC, Lake Pamvotis cluster).

Nitrospirae-like and *Acidobacteria*-like BacPamv sequences were difficult to be phylogenetically affiliated into the general bacterial phylogenetic tree, mainly due to their low homologies to known Nitrospirae and Acidobacterial sequences (sequence identity 89% - 96%) [53]. Therefore, two separate phylogenetic trees were constructed, one for *Acidobacteria*-like sequences (**Figure 2(b)**) and one for *Nitrospirae*-like ones (**Figure 2(c)**).

Overall, we detected 13 bacterial phyla in Lake Pamvotis sediments. *Proteobacteria*, *Bacteroidetes*, *Planctomycetes*, *Actinobacteria*, *Firmicutes*, *Acidobacteria* and *Nitrospirae*, have also been observed in other lakes and rivers [54] [55] [56]. The phylum of *Proteobacteria* was dominant in our sediment samples. This finding is highly reminiscent of the bacterial community structure in other lakes worldwide such as in Lake Taihu and in Lake Geneva [32] [41].

3.4. Diversity of the Dominant Archaeal Phylotypes in Lake Pamvotis Sediments

Relative to Bacteria, fewer DGGE bands were identified for Archaea (130 in to-

tal) but the banding pattern of Archaea was more variable (Figure S2) and a higher number of different archaeal DNA sequences were retrieved (92 in total). A phylogenetic tree of these sequences (ArcPamv, red symbols in Figure 3) is presented in Figure 3.

Nineteen of the 92 archaeal sequences (20.65%) were found to have <97% identity to any already known GenBank entry. When comparing with already known cultivated archaeal species, 88 of these sequences (95.65%) were found to have <97% identity to any sequence from cultured Archaea (Table S4). Sequences retrieved were mainly affiliated with *Euryarchaeota*. Only three of them were classified as *Miscellaneous Crenarchaeota* (MCG) (Figure 3).

Methanogenic Archaea of the *Methanomicrobiales*, *Methanocellales* and *Methanosarcinales* lineages were predominant in our samples, suggesting that the main archaeal metabolic function in the surface sediment of Lake Pamvotis is methane production. These lineages are frequently observed in the superficial zone of freshwater sediments [41] [57] [58] [59] [60].

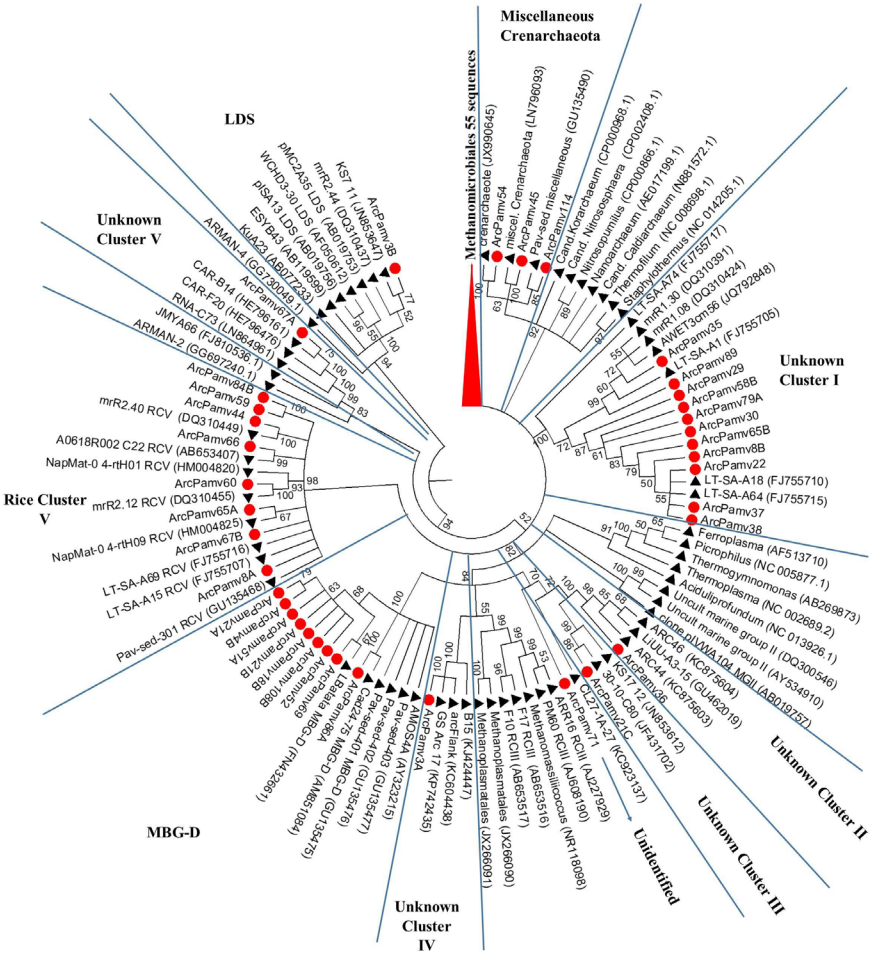


Figure 3. Distance tree based on the alignment of archaeal 16S rDNA sequences from Lake Pamvotis sediments (● ArcPamv) and (▲) a number of sequences with the highest similarities retrieved from GenBank/EMBL/DDBJ databases (Branches with bootstrap values below 50% have been deleted in this presentation).

Uncultured archaeal lineages appear to be ubiquitous in Lake Pamvotis as also observed in other freshwater sediments. Interestingly, we found that the phylogenetic cluster containing the most ArcPamv phylotypes coincides with a previously reported [41] "unknown", "uncharacterized" cluster (**Figure 3**, "unknown cluster I") (**Table S5**).

The numbers of ArcPamv sequences belonging to the Marine Benthic Group-D (MBG-D) and Rice Cluster V (RC-V) are comparable to those in the "unknown cluster I". MBG-D represents a highly common fraction of the prokaryotic community in hypersaline sediments and along with RC-V and Lake Dagow Sediment (LDS) lineages represents the most widely distributed uncultured lineages in freshwater sediments [61]. RC-V representatives from Lake Pamvotis form a robust clade with other RC-V sequences retrieved from lake sediments [41], rivers [62] and volcano mats [63] all over the world. Rice cluster V might correspond to non-methanogenic anaerobic Archaea [41] [64]. It has been shown earlier that RC-V and, to a lesser extent, LDS display pronounced genetic diversity and are characterized by long phylogenetic branches [61]. This also holds true for our phylogenetic analysis (**Figure 3**).

Based on our phylogenetic tree, the LDS cluster was revealed to be more closely related to *Candidatus Parvarchaeum acidiphilum* (ARMAN-4) [65]. Moreover, the rare "unknown cluster V" was found to be related to *Micrarchaeum acidiphilum* (ARMAN-2) [64] [66]. Thus, it is tempting to speculate on the physiology and ecology of these clusters, especially for the LDS cluster which is common in freshwater sediments [67] [68].

ARMANS, are nanosized Archaea which have been discovered in chemoautotrophic biofilms of the acidic metal rich Richmond Mine of Iron Mountain California [65]. ARMANS live in association with *Thermoplasmatales* and contain split genes and high AT contents [65] which are typical of fast evolving symbionts.

Could LDS or the "unknown cluster V" represent acidophilic nanosized symbionts of archaeal lineages related to *Thermoplasmatales*? This remains to be elucidated. Based on the available 16S rDNA fragments, the representatives of both the LDS and the "unknown cluster V" are characterized by high AT contents comparable to the ones of ARMANS.

The four other Euryarchaeotal rare sequences (ArcPamv36, ArcPamv21C, ArcPamv71 and ArcPamv3A) were found to be related to *Thermoplasmatales*. Finally, three archaeal sequences (ArcPamv54, ArcPamv45 and ArcPamv114) fall into three robust closely related but distinct clusters with external sequences which have been previously characterized as *Miscellaneous Crenarchaeota Group* (MCG) [35] [69]. In our phylogenetic analysis these MCG clusters were found to be more closely related to *Korarchaeota*/*Thaumarchaeota*. This is in accordance with previously published studies emphasizing that the affiliation of MCG and MBG-B within the *Crenarchaeota* is debated and proposing an alternative phylogenetic relationship either to *Thaumarchaeota* or to the *Aigarchaea* [70] [71].

In any case, MCG is a cosmopolitan group, frequently identified in anoxic habitants [42] [72]. Members of the MCG cluster are considered as heterotrophic anaerobes [73] and suggestively, they may obtain energy from the anaerobic oxidation of methane [73] in buried sediments. MCGs were found to be predominant in the intermediate layers of Lake Pavin sediments and their abundance was correlated with the decrease of methane concentrations in these layers [35]. In our study, the low number of MCG sequences retrieved could be attributed to the use of surface sediments only.

3.5. Relations between Physicochemical Variables, Prokaryotic Abundances and Diversity of the Dominant Prokaryotic Phylotypes

Regarding nutrient loads, TN was positively correlated with bacterial and, to a lesser extent, with archaeal abundances, whereas TOC was found to affect mainly the archaeal abundances (Table 3). These findings suggest that Bacteria are

Table 3. Results of correlation analysis between physicochemical and biological variables.

	Bacteria	Archaea
Depth	0.29	0.34
T	−0.34	0.07
pH	−0.57	−0.85
TC	0.12	0.55
TOC	0.27	0.65
TP	0.44	0.01
TN	0.61	0.54
Na	−0.02	0.39
K	0.56	0.54
Ca	0.58	0.75
Mg	0.54	0.64
Cl	0.16	0.59
SO_4	0.46	0.43
Sb	−0.53	−0.61
Ni	−0.69	−0.91
Hg	−0.32	0.07
Mn	−0.02	−0.08
Fe	−0.46	−0.52
Cu	0.40	0.19
Cr	−0.66	−0.81
Zn	0.20	0.09
As	0.55	0.80

Results of correlation analysis between physicochemical variables and bacterial/archaeal abundances. Spearman's correlation coefficients are shown. Statistically significant correlations are indicated in yellow ($p < 0.05$) or in red ($p < 0.001$).

the major players in the recycling of nitrogen and Archaea might be more important for carbon mineralization. In SS2, which is more heavily loaded with TC and TOC, methanogenic phylotypes are more common than in SS1 (**Table S5**). In any case, key functional genes, of both bacterial and archaeal origin, involved in nitrogen and carbon metabolism need to be studied in order to address this hypothesis more rigorously [74] [75].

Calcium concentration levels were correlated positively with both bacterial and archaeal cell numbers, suggesting a possible adaptation of the prokaryotic populations to a calcareous environment. Such an environment has been established in the sediments of the lake from ancient years, since the surrounding mountains consist mainly of lime bedrocks.

Concerning heavy metals, As had a strong positive effect on archaeal and a mild positive effect on bacterial cell abundances. In contrast, Ni and Cr seem to affect negatively both bacterial and archaeal abundances and, again, the effect is stronger on Archaea. Given that genes for metabolism, resistance and detoxification of metals are widespread throughout the archaeal and the bacterial domains [76] [77] [78] the contrasting effects of As and Ni or Cr on prokaryotic abundances in Lake Pamvotis are puzzling. One possible explanation for the positive effect of As is probably the time of exposure. It seems likely that the prokaryotic populations have coped with As for a longer time period compared to Ni and Cr and this has leaded to an adaptation of both Bacteria and Archaea to As contamination. Indeed, this should have been the case at least for Ni, since high Ni concentrations have been measured only during the last ten years. Moreover, it is of interest that Ni exceeds PEC in the sediments of the Lake and Cr exceeds TEC during all seasons, whereas As does not exceed either PEC or TEC (**Table 1**). The higher amounts of both Ni and Cr in SS1 might explain the lower abundances of both Bacteria and Archaea in this station compared to SS2.

In our study, pH was found to affect negatively the abundances of both Archaea and Bacteria, but the most significant effect was found for Archaea (**Table 3**).

Soil pH affects the chemical form, concentration and availability of different substrates [79]. The pH affects also methanogenesis. At slightly acidic conditions (pH 6.5) acetoclastic methanogenesis is inhibited. In contrast hydrogenotrophic methanogenesis is affected only slightly (0.03% compared to control pH 7.0) [80]. Given that most of the archaeal phylotypes isolated in our study are related to *Methanogens*, the higher abundances of Archaea in the station with the lower pH could be attributed to the prevalence of hydrogenotrophic methanogens in this station. Interestingly, most of the sequences related to *Methanoregula boonei* [81], an exclusively hydrogenotrophic archaeon were isolated from SS2. Based also on our results (**Table S5**), representatives of RC-V are more abundant in SS2 than in SS1 and since RC-V are thought not to be methanogenic, we may conclude that there is not a simple negative relationship between pH and the abundance of sedimental archaeal communities.

Concerning diversity, there are no obvious differences between the two sample stations with respect to the dominant bacterial phylotypes (**Table S3**). In contrast, with respect to Archaea, the numbers of different methanogenic and RC-V representatives are higher in SS2 than in SS1 (**Table S5**). Overall, the archaeal diversity in Lake Pamvotis sediments appears to be higher than the bacterial diversity at least for the dominant phylotypes.

From the relatively limited available literature, numerical differences between bacterial and archaeal diversities in lake sediments remain unclear. Some previously published studies indicate a higher bacterial over archaeal diversity in lake sediments [41] [82] while others point to a higher diversity of Archaea [83] [84]. In view of our current evidence, traditional techniques combined with next-generation sequencing technology can theoretically illustrate the overall diversity [85] [86] [87] and such studies should be important for an in-depth analysis of the community structure in the sediments of Lake Pamvotis.

A number of environmental factors such as pH [88], and heavy metals [89] were recognized as important determinants of prokaryotic community structure in previous studies. Given that there are differences in the determined environmental parameters between the two sampling stations in Lake Pamvotis, the essentially equal numbers of different bacterial sequences in the two stations suggest that the bacterial community diversity is not sensitive to these environmental factors. In contrast, the archaeal diversity was clearly greater in SS2 (**Table S5**). Lower pH values, lower concentrations of Ni and Cr along with higher As and TC concentrations are the main environmental factors differentiating SS2 from SS1; these factors are potential determinants of the archaeal diversity. Significant decrease in microbial diversity due to metal contamination was shown previously for Archaea in other lake sediments [33]. Moreover, in high As shallow aquifers, the increase of As concentrations apparently shifts the dominant archaeal populations from *Thaumarchaeota* to *Euryarchaeota* (mainly methanogens) [90]. Based on the literature and our data, we postulate that the decreased archaeal diversity in SS1 compared to SS2 could be attributed to the presence of higher amounts of Ni and Cr in this station. Consequently, it seems likely that a combination of the higher amounts of As and TC and the lower pH values in SS2 (**Table 1**) could be responsible for the higher diversity of both *Methanogens* and RC-V representatives in this station.

4. Conclusions

To our knowledge, this is the first study on both bacterial and archaeal abundances, diversity and community structure in the sediments of an ancient lake within the major European freshwater biodiversity hotspot.

Ni and Cr affect negatively both bacterial and archaeal abundances while Ca concentrations were found to have a positive effect. pH affects negatively mainly the archaeal abundance. TN has a strong positive effect on bacterial abundance, whereas As and TOC affect mainly Archaea.

Based on molecular characterization of the microbial communities, several new prokaryotic species were identified. A new class of *Cyanobacteria* was discovered in Lake Pamvotis sediments and termed "Lake Pamvotis cluster" (LPC). Concerning Archaea, most of the sequences retrieved from the sediments were affiliated to *Euryarchaeota* (dominated by Methanogenic Archaea). Interestingly, the widespread uncultivated cluster LDS was found to be phylogenetically related to ARMAN-4 lineage suggesting an unprecedented ecological role for this cluster.

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Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary Material

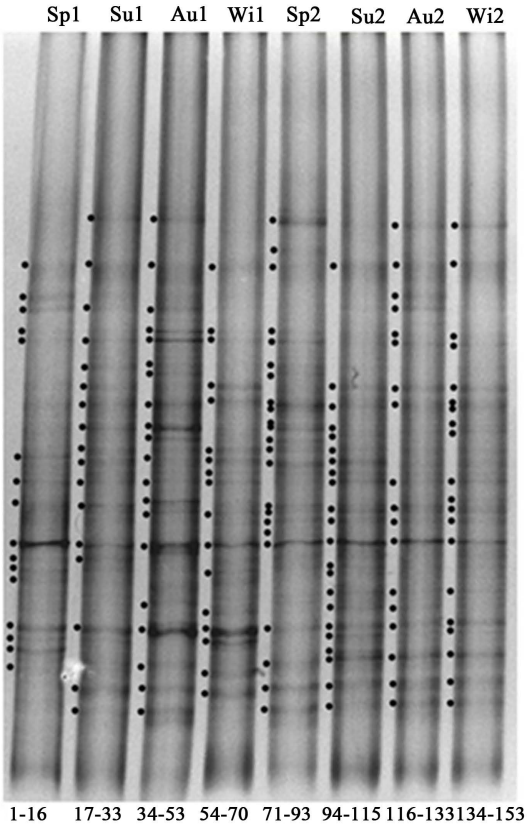

Figure S1. Bacterial species composition in the sediments of Lake Pamvotis as revealed by 16S rDNADGGE profiles. All dotted bands were excised, reamplified and sequenced. Sp: Spring, Su: Summer, Au: Autumn, Wi: Winter, 1: SS1, 2: SS2.

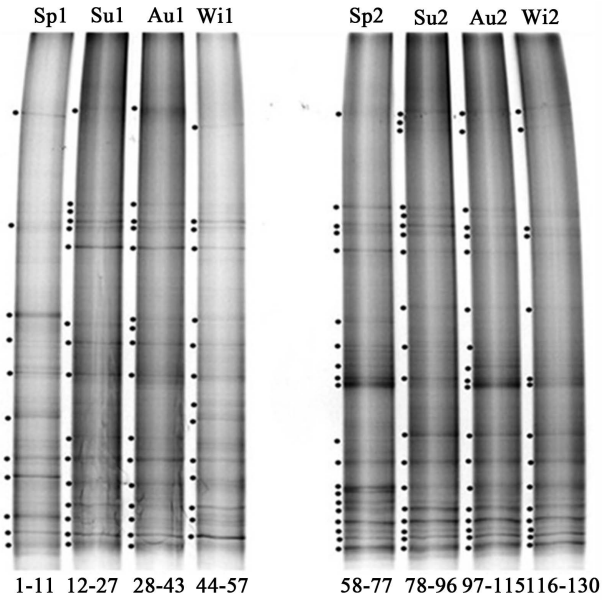

Figure S2. Archaeal species composition in the sediments of Lake Pamvotis as revealed by 16S rDNADGGE profiles. All dotted bands were excised, reamplified and sequenced. Sp: Spring, Su: Summer, Au: Autumn, Wi: Winter, 1: SS1, 2: SS2.

Clone Affiliation of Bacteria

Table S1. Clone affiliation of bacterial 16S rDNA sequences retrieved from Lake Pamvotis sediments, to known 16S rDNA sequences in public databases

Clone name	Accession number	% query, % identity culture collection	% query, % identity cultured species
BacPamv1	KP244158	100% 99% HM153665.1	100% 99% NR029024.1 *Hydrogenophaga defluvii* BSB 9.5
BacPamv2	KP244159	99% 98% FQ659268.1	99% 88% NR074757.1 *Treponema caldaria* DSM 7334
BacPamv3	KP244160	100% 93% GQ472421.1	100% 83% NR075001.1 *Moorella thermoacetica* ATCC 39073
BacPamv4	KP244161	100% 99% HM243914.1	100% 86% NR074330.1 *Nitrosococcus oceani* ATCC 19707
BacPamv5	KP244162	100% 96% JN805711.1	100% 92% NR075002.1 *Syntrophobacter fumaroxidans* MPOB
BacPamv6	KP244163	100% 87% JQ516335.1	100% 78% NR075009.1 *Geobacter sulfurreducens* PCA
BacPamv7	KP244164	100% 95% HQ910926.1	100% 85% NR028695.1 *Lewinella nigricans* SS-2
BacPamv8	KP244165	100% 99% KC432448.1	100% 86% NR104911.1 *Vampirovibrio chlorellavorus* ICPB 3707
BacPamv9A	KP244166	100% 99% KC432448.1	100% 85% NR043559.1 *Gracilibacter thermotolerans* JW/YJL-S1
BacPamv9B	KP244167	100% 100% JF265807.1	100% 96% NR029287.1 *Nitrospira moscoviensis* NSP M-1
BacPamv10	KP244168	100% 99% HM346679.1	100% 94% NR042824.1 *Collimonas arenae* NCCB 100031
BacPamv11	KP244169	100% 92% EF203209.1	100% 85% NR036977.1 *Thiococcus pfennigii* 4250
BacPamv12	KP244170	100% 99% AB661525.1	100% 84% NR109681.1 *Thermoanaerobaculum aquaticum* MP-01

Continued

BacPamv13A	KP244171	100% 99% AB196055.1	100% 92% NR044309.1 *Steroidobacter denitrificans* F5
BacPamv13B	KP244172	99% 93% HF677528.1	98% 85% NR075001.1 *Moorella thermoacetica* ATCC 39073
BacPamv13C	KP244173	100% 99% KC989704.1	100% 99% NR074314.1 *Microcystis aeroginosa* NIES-843
BacPamv14	KP244174	100% 90% JN473052.1	100% 78% NR075001.1 *Moorella thermoacetica* ATCC 39073
BacPamv15	KP244175	100% 99% HM346679.1	100% 93% NR042824.1 *Collimonas arenae* NCCB 100031
BacPamv16	KP244176	100% 99% JN868188.1	100% 92% NR043249.1 *Denitratisoma oestradiolicum* AcBE2-1
BacPamv17A	KP244177	98% 96% JQ583178.1	97% 94% NR074351.1 *Candidatus solibcter* Ellin 6076
BacPamv17B	KP244178	100% 96% HQ904418.1	100% 85% NR102459.1 *Chamaesiphon minutes* PCC 6605
BacPamv18	KP244179	100% 99% KF287757.1	100% 99% NR025816.1 *Porphyrobacter donghaensis* SW-132
BacPamv19	KP244180	100% 99% KC248046.1	100% 98% NR042941.1 *Paucibacter toxinivorans* 2C20
BacPamv20A	KP244181	100% 97% HQ661184.1	100% 84% NR102468.1 *Stanieria cyanospaera* PCC 7437
BacPamv20B	KP244182	100% 89% EU376186.1	100% 79% NR102456.1 *Leptolyngbya* PCC 7376
BacPamv21	KP244183	100% 99% EU104276.1	100% 89% NR040990.1 *Owenweeksia hongkongensis* UST 20020801
BacPamv22	KP244184	100% 96% HQ661184.1	100% 84% NR102456.1 *Leptolyngbya* PCC 7376
BacPamv23	KP244185	100% 96% KF939466.1	100% 94% NR102987.1 *Clostridium clariflavum* DSM 19732

Continued

BacPamv24A	KP244186	100% 99% KC541335.1	100% 93% NR044309.1 *Steroidobacter denitrificans* FS
BacPamv24B	KP244187	99% 97% AY693835.1	99% 83% NR025079.1 *Desulfomonile limimaris* DSB-M
BacPamv25	KP244188	100% 99% HM243891.1	100% 88% NR074345.1 *Thermodesulfovibrio yellowstonii* DSM 11347
BacPamv26	KP244189	100% 99% KC666549.1	100% 97% NR043993.1 *Rheinheimera tangshanensis* JA3-B52
BacPamv27	KP244190	100% 93% HQ246251.1	100% 93% NR029024.1 *Hydrogenophaga defluvii* BSB 9.5
BacPamv28	KP244191	100% 91% AB722172.1	100% 84% NR037137.1 *Treponema medium* G7201
BacPamv29	KP244192	99% 91% AB661540.1	99% 86% NR074757.1 *Treponema caldaria* DSM 7334
BacPamv30	KP244193	100% 99% JN257048.1	100% 95% NR074317.1 *Nostoc punctiforme* PCC 73102
BacPamv31	KP244194	100% 91% GQ356966.1	100% 84% NR025150.1 *Desulfobulbus mediterraneus* 86FS1
BacPamv32	KP244195	100% 99% KF556697.1	100% 98% NR074760.1 *Albidiferax ferrireducens* T118
BacPamv33	KP244196	100% 99% AB793710.1	100% 97% NR026102.1 *Clostridium papyrosolvens* DSM 2792
BacPamv34	KP244197	100% 92% GU208417.1	100% 85% NR028745.1 *Thioalkalivibrio denitrificans* ALJD
BacPamv35	KP244198	100% 89% AM181924.1	100% 84% NR043929.1 *Skermanella aerolata* 5416T-32
BacPamv36	KP244199	100% 99% HG792168.1	100% 99% NR036911.2 *Aeromonas media* RM
BacPamv37	KP244200	100% 99% KC815481.1	100% 99% NR102447.1 *Cyanobium gracile* PCC 6307

Continued

BacPamv38	KP244201	100% 99% LK054500.1	100% 99% NR075062.2 *Micrococcus luteus* NCTC 2665
BacPamv39	KP244202	100% 92% KF384384.1	100% 87% NR104682.1 *Marinilabilia salmonicolor* JCM 21150 NBRC 15946
BacPamv40	KP244203	100% 98% EU376186.1	100% 83% NR102456.1 *Leptolyngbya* PCC 7376
BacPamv41	KP244204	100% 93% DQ642331.1	100% 90% NR041306.1 *Syntrophorhabdus aromaticivorans* U1
BacPamv42	KP244205	100% 96% GU197631.1	100% 92% NR074317.1 *Nostoc punctiforme* PCC 73102
BacPamv43	KP244206	100% 88% AB186797.1	100% 81% NR043385.1 *Dictyoglomus turgidum* DSM 6724
BacPamv44	KP244207	100% 95% AB486150.1	100% 89% NR075011.1 *Geobacter metallireducens* GS-15
BacPamv45	KP244208	100% 99% KC432448.1	100% 85% NR043559.1 *Gracilibacter thermotolerans* JW/YJL-S1
BacPamv46	KP244209	100% 96% GU454906.1	100% 87% NR074757.1 *Treponema caldaria* DSM 7334
BacPamv47	KP244210	100% 99% KC432448.1	100% 84% NR043559.1 *Gracilibacter thermotolerans* JW/YJL-S1
BacPamv48	KP244211	99% 92% AB240355.1	99% 84% NR044075.1 *Thermodesulfovibrio hydrogeniphiles* HbrS
BacPamv49	KP244212	100% 93% JN397726.1	100% 91% NR028715.1 *Acidovorax temperans* PHL
BacPamv50	KP244213	100% 99% JX223096.1	100% 99% NR040800.1 *Vogesella indigofera* ATCC 19706
BacPamv51	KP244214	100% 96% JQ624950.1	100% 92% NR026102.1 *Clostridium papyrosolvens* DSM 2792

Clone affiliation of culturable Bacteria

Table S2. Sequence analysis of 16S rDNA sequences retrieved from cultured bacterial isolates from Lake Pamvotis sediments.

Clone name	Accession number	% query, % identity culture collection	% query, % identity cultured species
PamvBac iso.1	KU862661	100% 100% KF481602.1	100% 99% NR042502.1 *Massilia aurea* AP13
PamvBac iso.2	KU862662	100% 99% KF556686.1	100% 97% NR043699.1 *Rheinheimera chironomi* K19414
PamvBac iso.3	KU862663	100% 99% EF471218.1	100% 99% NR042596.1 *Cryseobacterium luteum* P456/04
PamvBac iso.4	KU862664	100% 99% JX223096.1	100% 99% NR040800.1 *Vogesella indigofera* ATCC 19706
PamvBac iso.5	KU862665	100% 99% KF556697.1	100% 98% NR114646.1 *Rhodoferax ferrireducens* T118
PamvBac iso.6	KU862666	100% 99% HG792168.1	100% 99% NR036911.2 *Aeromonas media* RM
PamvBac iso.7	KU862667	100% 99% KF555636.1	100% 99% NR041057.1 *Flavobacterium frigidimaris* KUC-1
PamvBac iso.8	KU862668	100% 99% JF145482.1	100% 99% NR044292.1 *Flavobacterium resistens* BD-b365
PamvBac iso.9	KU862669	100% 99% KC666807.1	100% 99% NR025425.1 *Acinetobacteria parvus* LUH 4616
PamvBac iso.10	KU862670	100% 100% JX657101.1	100% 98% NR108576.1 *Flavobacterium compostarboris* 15C3
PamvBac iso.11	KU862671	100% 99% KC666549.1	100% 97% NR043993.1 *Rheinheimera tangshanensis* JA3-B52
PamvBac iso.12	KU862672	100% 99% KC294042.1	100% 99% NR029319.1 *Pseudomonas anguilliseptica* S1
PamvBac iso.13	KU862673	100% 99% GU291856.1	100% 98% NR109728.1 *Flavobacterium cutihirudinis* E89
PamvBac iso.14	KU862674	97% 99% JQ317797.1	97% 98% NR029319.1 *Pseudomonas anguilliseptica* S1

Continued

PamvBac iso.15	KU862675	100% 99% HM149209.1	100% 99% NR044581.1 *Flavobacterium chungangense* CJ7
PamvBac iso.16	KU862676	99% 99% KF894688.1	99% 98% NR115957.1 *Chryseobacterium flavum* strain CW-E2
PamvBac iso.17	KU862677	100% 99% KC248046.1	100% 98% NR042941.1 *Paucibacter toxinivorans* 2C20
PamvBac iso.18	KU862678	100% 93% HQ246251.1	100% 93% NR029024.1 *Hydrogenophaga defluvii* BSB 9.5
PamvBac iso.19	KU862679	100% 100% KC294042.1	100% 100% NR029319.1 *Pseudomonas anguilliseptica* S1
PamvBac iso.20	KU862680	100% 99% HM153665.1	100% 99% NR029024.1 *Hydrogenophaga defluvii* BSB 9.5
PamvBac iso.21	KU862681	100% 99% NR109522.1	100% 99% NR109522.1 *Flavobacterium fontis* MIC 3010
PamvBac iso.22	KU862682	100% 99% KF287757.1	100% 99% NR025816.1 *Porphyrobacter donghaensis* SW-132
PamvBac iso.23	KU862683	100% 99% LK054500.1	100% 99% NR075062.2 *Micrococcus luteus* NCTC 2665

Table S3. Distribution of bacterial 16S rDNA clones in Lake Pamvotis sample stations.

	clone name	SS1	SS2	Sp.		Su.		Au.		Wi.	
				SS1	SS2	SS1	SS2	SS1	SS2	SS1	SS2
γ-Proteobacteria	BacPamv26	√	√	-	√	-	-	√	-	-	-
	BacPamv36	√	-	-	-	-	-	-	-	√	-
	BacPamv13A	√	-	√	-	-	-	-	-	-	-
	BacPamv24A	√	√	-	-	√	√	√	-	-	-
	BacPamv4	√	√	√	√	-	-	√	√	√	√
	BacPamv35	√	-	-	-	-	-	-	-	√	-
	BacPamv11	√	-	√	-	-	-	-	-	-	-
TOTAL SEQUENCES		**7**	**3**	**3**	**2**	**1**	**1**	**3**	**1**	**3**	**1**
β-Proteobacteria	BacPamv10	√	-	√	-	√	-	-	-	-	-
	BacPamv15	√	√	√	-	-	√	-	√	-	√
	BacPamv50	-	√	-	-	-	√	-	-	-	-
	BacPamv16	√	√	√	√	-	-	√	-	-	-

Continued

	BacPamv27	√	√	-	-	-	√	√	-	-	-
	BacPamv49	-	√	-	-	-	√	-	√	-	-
	BacPamv19	√	√	-	-	√	√	-	√	√	√
	BacPamv1	√	√	√	√	√	√	√	√	√	√
	BacPamv32	√	-	-	-	-	-	-	-	√	-
TOTAL SEQUENCES		**7**	**7**	**4**	**2**	**3**	**6**	**3**	**4**	**3**	**3**
α-Proteobacteria	BacPamv18	√	-	-	-	√	-	-	-	-	-
TOTAL SEQUENCES		**1**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
Bacteroidetes	BacPamv7	√	√	√	-	√	√	√	√	√	√
	BacPamv21	√	-	-	-	√	-	√	-	-	-
	BacPamv39	-	√	-	√	-	-	-	-	-	√
TOTAL SEQUENCES		**2**	**2**	**1**	**1**	**2**	**1**	**2**	**1**	**1**	**2**
δ-Proteobacteria	BacPamv24B	√	√	-	√	√	√	-	√	-	√
	BacPamv44	-	√	-	√	-	√	-	√	-	√
	BacPamv41	-	√	-	√	-	-	-	-	-	√
TOTAL SEQUENCES		**1**	**3**	**0**	**3**	**1**	**2**	**0**	**2**	**0**	**3**
Actinobacteria	BacPamv38	-	√	-	√	-	-	-	-	-	-
TOTAL SEQUENCES		**0**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Gemmatimonadetes	BacPamv34	√	√	-	-	-	√	-	-	√	-
TOTAL SEQUENCES		**1**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**1**	**0**
Spirochaetes	BacPamv2	√	√	√	-	-	-	-	√	-	-
	BacPamv29	√	-	-	-	-	-	√	-	-	-
	BacPamv28	√	√	-	√	-	-	√	-	-	-
TOTAL SEQUENCES		**3**	**2**	**1**	**1**	**0**	**0**	**2**	**1**	**0**	**0**
Planctomycetes	BacPamv14	√	√	√	-	-	√	-	-	√	√
	BacPamv6	√	√	√	√	-	√	√	-	√	-
TOTAL SEQUENCES		**2**	**2**	**2**	**1**	**0**	**2**	**1**	**0**	**2**	**1**
Cyanobacteria	BacPamv20A	√	√	-	√	√	-	√	-	-	-
	BacPamv40	-	√	-	√	-	√	-	-	-	√
	BacPamv22	√	√	-	√	√	√	-	-	√	-
	BacPamv20B	-	√	-	√	-	√	-	√	-	√
	BacPamv17B	-	√	-	√	-	-	-	√	-	√
	BacPamv30	√	-	-	-	-	-	√	-	√	-
	BacPamv42	-	√	-	√	-	-	-	-	-	-
	BacPamv37	√	-	-	-	-	-	-	-	√	-
	BacPamv13C	√	√	-	√	-	√	√	√	√	√

Continued

TOTAL SEQUENCES		**5**	**7**	**0**	**7**	**2**	**4**	**3**	**3**	**4**	**4**
Firmicutes	BacPamv33	√	√	-	-	-	√	-	-	√	-
	BacPamv51	-	√	-	-	-	-	-	-	-	√
	BacPamv23	√	-	-	-	√	-	-	-	-	-
TOTAL SEQUENCES		**2**	**2**	**0**	**0**	**1**	**1**	**0**	**0**	**1**	**1**
Nitrospirae	BacPamv25	√	√	-	√	√	√	√	√	-	√
	BacPamv31	√	-	-	-	-	-	√	-	-	-
	BacPamv48	-	√	-	-	-	√	-	√	-	√
	BacPamv12	√	-	√	-	-	-	-	-	-	-
	BacPamv9B	√	-	√	-	√	-	-	-	-	-
TOTAL SEQUENCES		**4**	**2**	**2**	**1**	**2**	**2**	**2**	**2**	**0**	**2**
Acidobacteria	BacPamv5	√	√	√	√	√	-	√	√	√	√
	BacPamv3	√	√	√	-	√	-	√	√	-	-
	BacPamv13B	√	-	-	-	√	-	√	-	√	-
	BacPamv17A	√	√	-	√	√	-	-	√	-	-
TOTAL SEQUENCES		**4**	**3**	**2**	**2**	**4**	**0**	**3**	**3**	**2**	**1**
Unclassified cluster I	BacPamv43	-	√	-	√	-	√	-	√	-	√
TOTAL SEQUENCES		**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**
Unclassified cluster II	BacPamv45	-	√	-	√	-	-	-	-	-	-
	BacPamv47	-	√	-	-	-	√	-	-	-	-
	BacPamv9A	√	√	√	√	-	√	√	√	√	√
	BacPamv8	√	-	√	-	√	-	-	-	-	-
TOTAL SEQUENCES		**2**	**3**	**2**	**2**	**1**	**2**	**1**	**1**	**1**	**1**
Unclassified cluster III	BacPamv46	-	√	-	-	-	√	-	-	-	-
TOTAL SEQUENCES		**0**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
	SS1	**SS2**									
TOTAL SEQUENCES	**41**	**40**									

SS1: Sample Station 1

SS2: Sample Station 2

Sp.: Spring

Su.: Summer

Au.: Autumn

Wi.: Winter

Clone Affiliation of Archaea

Table S4. Clone affiliation of archaeal 16S rDNA sequences retrieved from Lake Pamvotis sediments, to known 16S rDNA sequences from public databases.

Clone name	Accession number	% query, % identity culture collection	% query, % identity cultured species
ArcPamv1	KC510289	100% 96% JN617408	100% 95% NR028163 *Methanolinea tarda* NOBI-1
ArcPamv2	KC510290	100% 99% JN617359	100% 95% NR 044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv3A	KC510291	100% 94% JX196214	100% 79% NR028646 *Methanotorris formicicus* strain Mc-S-70
ArcPamv3B	KC510292	99% 89% JN853647	100% 72% NR029140 *Methanococcus aeolicus* Nankai-3
ArcPamv4A	KC510293	100% 99% HM244131	100% 95% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv4B	KC510294	100% 99% JQ795001	100% 80% NR042784 *Methanobrevibacter ruminantium* strain M1
ArcPamv4C	KC510295	100% 99% JX426833	100% 96% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv5	KC510296	100% 99% JN 617444	100% 97% NR028242 *Methanosaeta concilii* strain Opfikon
ArcPamv6	KC510297	100% 98% DQ301909	100% 95% NRO44422 *Methanosphaerula palustris* strain E1-9c
ArcPamv7	KC510298	99% 99% JQ794950	99% 97% NR028163 *Methanolinea tarda* NOBI-1
ArcPamv8A	KC510299	100% 99% JF431625	100% 78% NR029059 *Palaeococcus helgesonii* strain PI1
ArcPamv8B	KC510300	100% 99% FJ755715	88% 95% NR028179 *Thermococcus thioreducens* OGL-20P
ArcPamv9	KC510301	100% 99% JQ245676	100% 94% NR028163 *Methanolinea tarda* NOBI-1
ArcPamv10	KC510302	100% 99% EF639431	100% 96% NR028163 *Methanolinea tarda* NOBI-1

Continued

ArcPamv11	KC510303	100% 99 JX426833	100% 96% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv12	KC510304	99% 99% AY125724	99% 96% NR028163 *Methanolinea tarda* NOBI-1
ArcPamv13	KC510305	99% 99% DQ785302	99% 95% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv14	KC510306	100% 99% HQ330724	100% 95% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv15	KC510307	100% 98% HQ330702	100% 91% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv16	KC510308	100% 99% JQ794997	100% 96% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv17	KC510309	100% 99% AM503280	100% 95% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv18A	KC510310	100% 98% EF639431	100% 96% NR028163 *Methanolinea tarda* NOBI-1
ArcPamv18B	KC510311	100% 99% JX426828	100% 80% NR042784 *Methanobrevibacter ruminantium* M1 strain M1
ArcPamv20	KC510312	100% 99% FM165672	100% 96% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv21A	KC510313	100% 99% JQ795001	100% 80% NR042784 *Methanobrevibacter ruminantium* M1 strain M1
ArcPamv21B	KC510314	100% 99% JQ794995	100% 80% NR042784 *Methanobrevibacter ruminantium* M1 strain M1
ArcPamv21C	KC510315	100% 99% JF431702	100% 78% NR029055 *Thermococcus aegaeus*
ArcPamv22	KC510316	100% 99% FJ755715	85% 80% NR028179 *Thermococcus thioreducens* OGL-20P
ArcPamv23	KC510317	100% 99% HM244131	100% 95% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv24	KC510318	100% 99% HQ330702	100% 93% NR044422 *Methanosphaerula palustris* strain E1-9c

Continued

ArcPamv25	KC510319	100% 99% JQ245676	100% 94% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv28	KC510320	100% 99% DQ676243	100% 95% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv29	KC510321	100% 99% HQ330690	90% 92% NR042740 *Thermococcus hydrothermalis* strain AL662
ArcPamv30	KC510322	100% 99% HQ330690	85% 81% NR028179 *Thermococcus thioreducens* OGL-20P
ArcPamv31	KC510323	100% 99% DQ785302	100% 94% NR028163 *Methanolinea tarda* NOBI-1
ArcPamv33	KC510324	100% 98% JF262336	100% 96% NR028163 *Methanolinea tarda* NOBI-1
ArcPamv35	KC510325	100% 97% JQ792848	100% 78% NR028248 *Methanothermobacter defluvii*
ArcPamv36	KC510326	100% 92% JF853612	100% 78% NR043089 *Methanomethylovorans thermophila*
ArcPamv37	KC510327	100% 99% FJ755715	90% 92% NR028179 *Thermococcus thioreducens* OGL-20P
ArcPamv38	KC510328	100% 100% FJ755715	90% 92% NR028179 *Thermococcus thioreducens* OGL-20P
ArcPamv39	KC510329	100% 99% JQ079951	100% 98% NR028242 *Methanosaeta concilii* strain Opfikon
ArcPamv42	KC510330	99% 99% JQ794950	99% 97% NR028163 *Methanolinea tarda* NOBI-1
ArcPamv43	KC510331	99% 99% JX426879	99% 96% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv44	KC510332	99% 98% LN896671	99% 76% NR029140 *Methanococcus aeolicus* NanKai-3
ArcPamv45	KC510333	94% 98% AJ240005 97% 96% AF005766	94% 100% NR029214 *Thermofilum pendens* strain Hvv3, DSM 2474 94% 100% NR028877 *Staphylothermus hellenicus* DSM 12710 strain P8

Continued

ArcPamv49	KC510334	100% 98% JF980361	100% 95% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv51A	KC510335	100% 99% JF431901	100% 80% NR042784 *Methanobrevibacter ruminantium* M1 strain M1
ArcPamv51B	KC510336	99% 98% JQ794997	99% 94% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv52	KC510337	100% 99% HM244091	100% 80% NR042784 *Methanobrevibacter ruminantium* M1 strain M1
ArcPamv54	KC510338	100% 99% JF431775	99% 83% NR043512 *Ignisphaera aggregans* DSM 17230 strain AQ1.S1
ArcPamv55	KC510339	100% 99% FM165672	100% 96% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv57	KC510340	99% 99% JQ794950	99% 96% NR028163 *Methanolinea tarda* NOBI-1
ArcPamv58A	KC510341	99% 99% JN649164	99% 95% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv58B	KC510342	99% 96% FN432722	99% 78% NR042734 *Thermococcus barophilus* MP strain DSM 11836
ArcPamv59	KC510343	99% 97% HQ330736	100% 78% NR025718 *Methanococcus vannielii* strain 5B
ArcPamv60	KC510344	100% 99% DQ310455	100% 77% NR028210 *Ferroplasma cupricumulans* BH2
ArcPamv65A	KC510345	100% 90% HM004825	100% 78% NR029140 *Methanococcus aeolicus* NanKai-3
ArcPamv65B	KC510346	100% 97% FJ755715	100% 78% NR042781 *Methanobacterium bryantii* strain MOH
ArcPamv66	KC510347	98% 93% AB653407	100% 78% NR041513 *Thermogymnomonas acidicola* strain JCM 13583
ArcPamv67A	KC510348	100% 99% HE796161	100% 78% NR028701 *Methanocaldococcus vulcanius* M7
ArcPamv67B	KC510349	100% 99% HQ404340	100% 77% NR028646 *Methanotorris formicicus* strain Mc-S-70

Continued

ArcPamv69	KC510350	100% 99% JN853654	100% 80% NR042784 *Methanobrevibacter ruminantinum* M1
ArcPamv70	KC510351	99% 100% AB652545	100% 93% NR028164 *Methanocella paludicola* SANAE
ArcPamv71	KC510352	100% 90% EF639526	100% 80% NR044786 *Methanobrevibacter smithii* ATCC 35061
ArcPamv72A	KC510353	100% 98% JQ595987	100% 95% NR042789 *Methanospirillum hungatei* JF-1, strain JF1
ArcPamv72B	KC510354	100% 99% DQ785302	100% 94% NR044422 *Methanosphaerula palustris* strain E1-9c
ArcPamv75	KC510355	100% 96% JN649130	100% 94% NR043961 *Methanoculleus receptaculi*
ArcPamv76	KC510356	100% 99% AB775723	100% 97% NR028163 *Methanolinea tarda* NOBI-1
ArcPamv77A	KC510357	100% 99% JQ794950	100% 96% NR028163 *Methanolinea tarda* NOBI-1
ArcPamv77B	KC510358	99% 97% FN646492	99% 92% NR044422 *Methanosphaerula palustris* E1-9c
ArcPamv79A	KC510359	100% 96% JN853749	100% 78% NR029059 *Palaeococcus helgesonii*
ArcPamv79B	KC510360	100% 99% JX426833	100% 96% NR044422 *Methanosphaerula palustris* E1-9c
ArcPamv79C	KC510361	100% 99% JQ792430	100% 96% NR044422 *Methanosphaerula palustris* E1-9c
ArcPamv79D	KC510362	97% 99% HQ330660	97% 95% NR044422 *Methanosphaerula palustris* E1-9c
ArcPamv82	KC510363	100% 99% JX426833	100% 96% NR044422 *Methanosphaerula palustris* E1-9c
ArcPamv83A	KC510364	99% 98% EF639443	99% 93% NR044422 *Methanosphaerula palustris* E1-9c

Continued

ArcPamv83B	KC510365	100% 99% JQ245676	100% 94% NR028163 *Methanolinea tarda* NOBI-1
ArcPamv84A	KC510366	100% 99% JQ245676	100% 94% NR0128163 *Methanolinea tarda* NOBI-1
ArcPamv84B	KC510367	87% 87% HQ330736 97% 76% EU983178	82% 76% NR102915 *Methanothermococcus okinawensis* IH 1
ArcPamv86A	KC510368	100% 99% JN617381	100% 80% NR074217 *Aciduliprofundum boonei* T469 strain T469
ArcPamv86B	KC510369	99% 97% EU519275	99% 95% NR044422 *Methanosphaerula palustris* E1-9c
ArcPamv88	KC510370	98% 99% JF431886	98% 98% NR028242 *Methanosaeta concilii* strain Opfikon
ArcPamv89	KC510371	100% 96% JQ792848	100% 78% NR116289 *Methanobacterium movens* strain TS-2
ArcPamv90	KC510372	100% 97% FN646483	100% 93% NR028242 *Methanosaeta concilii* Opfikon
ArcPamv92	KC510373	100% 99% JQ079951	100% 98% NR028242 *Methanosaeta concilii* Opfikon
ArcPamv96	KC510374	100% 99% HE964957	100% 94% NR028242 *Methanosaeta concilii* Opfikon
ArcPamv108A	KC510375	100% 99% HQ330667	100% 94% NR044422 *Methanosphaerula palustris* E1-9c
ArcPamv108B	KC510376	99% 99% JX426828	99% 80% NR042784 *Methanobrevibacter ruminantium* M1
ArcPamv109	KC510377	100% 99% HQ330667	100% 95% NR044422 *Methanosphaerula palustris* E1-9c
ArcPamv112	KC510378	100% 99% HQ330702	100% 93% NR044422 *Methanosphaerula palustris* E1-9c
ArcPamv114	KC510379	100% 99% HM244128	100% 84% NR028877 *Staphylothermus hellenicus* DSM 12710 strain P8
ArcPamv115	KC510380	99% 98% FN646492	99% 92% NR04442 *Methanosphaerula palustris* E1-9c

Table S5. Distribution of archaeal 16S rDNA clones in Lake Pamvotis sample stations.

	clone name	SS1	SS2	Sp.		Su.		Au.		Wi.	
				SS1	SS2	SS1	SS2	SS1	SS2	SS1	SS2
Methanomicrobiales	ArcPamv9	√	√	√	√	√	√	√	√	√	√
	ArcPamv84A	-	√	-	-	-	√	-	-	-	-
	ArcPamv25	√	√	-	-	√	√	-	-	-	-
	ArcPamv83B	-	√	-	-	-	√	-	√	-	-
	ArcPamv13	√	√	-	√	√	√	√	√	-	-
	ArcPamv31	√	√	-	-	-	-	√	√	√	-
	ArcPamv72B	-	√	-	√	-	-	-	-	-	-
	ArcPamv58A	-	√	-	√	-	√	-	√	-	√
	ArcPamv33	√	√	-	√	-	-	√	-	√	-
	ArcPamv49	√	-	-	-	-	-	-	-	√	-
	ArcPamv28	√	-	-	-	-	-	√	-	-	-
	ArcPamv75	-	√	-	√	-	√	-	√	-	√
	ArcPamv77B	-	√	-	√	-	-	-	-	-	-
	ArcPamv115	-	√	-	-	-	-	-	√	-	-
	ArcPamv51B	√	-	-	-	-	-	-	-	√	-
	ArcPamv16	√	√	-	√	√	√	√	√	√	√
	ArcPamv4C	√	√	√	√	-	√	-	√	√	-
	ArcPamv79B	-	√	-	-	-	√	-	-	-	-
	ArcPamv43	√	√	-	-	-	-	√	√	-	-
	ArcPamv11	√	√	√	√	√	√	√	√	-	√
	ArcPamv82	-	√	-	-	-	√	-	-	-	-
	ArcPamv6	√	-	√	-	-	-	-	-	-	-
	ArcPamv14	√	√	-	-	√	√	-	-	-	-
	ArcPamv79D	-	√	-	-	-	√	-	-	-	-
	ArcPamv108A	-	√	-	-	-	-	-	√	-	-
	ArcPamv109	-	√	-	-	-	-	-	√	-	-
	ArcPamv23	√	√	-	√	√	√	√	√	-	√
	ArcPamv4A	√	√	√	√	√	√	√	√	√	-
	ArcPamv17	√	√	-	√	√	√	√	√	√	-
	ArcPamv55	√	-	-	-	-	-	-	-	√	-
	ArcPamv20	√	√	-	√	√	√	√	-	-	-
	ArcPamv2	√	-	√	-	-	-	-	-	-	-
	ArcPamv79C	-	√	-	-	-	√	-	-	-	-
	ArcPamv18A	√	-	-	-	√	-	√	-	-	-
	ArcPamv86B	-	√	-	-	-	√	-	-	-	-

Continued

	ArcPamv76	-	√	-	√	-	-	-	-	-	-
	ArcPamv7	√	√	√	√	√	√	√	√	√	√
	ArcPamv12	√	-	-	-	√	-	-	-	-	-
	ArcPamv42	√	-	-	-	-	-	√	-	-	-
	ArcPamv57	√	-	-	-	-	-	-	-	√	-
	ArcPamv10	√	√	√	√	√	√	√	√	√	√
	ArcPamv77A	-	√	-	√	-	-	-	-	-	-
	ArcPamv1	√	-	√	-	-	-	-	-	-	-
	ArcPamv83A	-	√	-	-	-	√	-	-	-	-
	ArcPamv24	√	√	-	√	√	√	√	√	√	√
	ArcPamv15	√	√	-	√	√	√	√	√	√	√
	ArcPamv12	√	-	-	-	√	-	-	-	-	-
	ArcPamv72A	-	√	-	√	-	-	-	-	-	-
	ArcPamv90	-	√	-	-	-	√	-	-	-	-
	ArcPamv70	-	√	-	√	-	-	-	√	-	-
	ArcPamv96	-	√	-	-	-	√	-	-	-	-
	ArcPamv5	√	√	√	√	√	√	√	√	√	√
	ArcPamv92	-	√	-	-	-	√	-	√	-	√
	ArcPamv39	√	-	-	-	-	-	√	-	-	-
	ArcPamv88	-	√	-	-	-	√	-	√	-	-
TOTAL SEQUENCES		**32**	**42**	**10**	**23**	**18**	**30**	**20**	**24**	**16**	**12**
Miscellaneous Crenarchaeota	ArcPamv54	√	-	-	-	-	-	-	-	√	-
	ArcPamv45	√	-	-	-	-	-	-	-	√	-
	ArcPamv114	-	√	-	-	-	-	-	√	-	-
TOTAL SEQUENCES		**2**	**1**	**0**	**0**	**0**	**0**	**0**	**1**	**2**	**0**
Unknown cluster I	ArcPamv35	√	-	-	-	-	-	√	-	-	-
	ArcPamv89	-	√	-	-	-	√	-	√	-	√
	ArcPamv29	√	-	-	-	-	-	√	-	-	-
	ArcPamv58B	-	√	-	√	-	√	-	-	-	-
	ArcPamv79A	-	√	-	-	-	√	-	-	-	-
	ArcPamv30	√	-	-	-	-	-	√	-	-	-
	ArcPamv65B	-	√	-	√	-	-	-	-	-	-
	ArcPamv8B	√	-	√	-	-	-	-	-	√	-
	ArcPamv22	√	-	-	-	√	-	√	-	-	-
	ArcPamv37	√	-	-	-	-	-	√	-	-	-
	ArcPamv38	√	√	-	√	-	√	√	-	-	-

Continued

TOTAL SEQUENCES		**7**	**5**	**1**	**3**	**1**	**4**	**6**	**1**	**1**	**1**
Unknown cluster II	ArcPamv36	√	-	-	-	-	-	√	-	-	-
TOTAL SEQUENCES		**1**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**
Unknown cluster III	ArcPamv21C	√	-	-	-	√	-	√	-	-	-
TOTAL SEQUENCES		**1**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**0**	**0**
Unidentified	ArcPamv71	-	√	-	√	-	-	-	-	-	-
TOTAL SEQUENCES		**0**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Unknown cluster IV	ArcPamv3A	√	-	√	-	-	-	-	-	-	-
TOTAL SEQUENCES		**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
MBG-D	ArcPamv86A	-	√	-	-	-	√	-	√	-	√
	ArcPamv69	-	√	-	√	-	-	-	-	-	-
	ArcPamv52	√	-	-	-	-	-	-	-	√	-
	ArcPamv108B	-	√	-	-	-	-	-	√	-	-
	ArcPamv18B	√	-	-	-	√	-	-	-	-	-
	ArcPamv21B	√	√	-	√	√	-	√	-	-	-
	ArcPamv51A	√	-	-	-	-	-	-	-	√	-
	ArcPamv4B	√	-	√		√	-	√	-	-	-
	ArcPamv21A	√	√	-	√	√	-	√	-	-	-
TOTAL SEQUENCES		**6**	**5**	**1**	**3**	**4**	**1**	**3**	**2**	**2**	**1**
Rice cluster V	ArcPamv8A	√	-	√	-	-	-	-	-	-	-
	ArcPamv67B	-	√	-	√	-	-	-	√	-	-
	ArcPamv65A	-	√	-	√	-	-	-	√	-	-
	ArcPamv60	-	√	-	√	-	-	-	-	-	-
	ArcPamv66	-	√	-	√	-	-	-	-	-	-
	ArcPamv44	√	√	-	-	-	√	-	√	√	√
	ArcPamv59	-	√	-	√	-	-	-	-	-	-
	ArcPamv84B	-	√	-	-	-	√	-	-	-	-
TOTAL SEQUENCES		**2**	**7**	**1**	**5**	**0**	**2**	**0**	**3**	**1**	**1**
Unknown cluster V	ArcPamv67A	-	√	-	√	-	-	-	√	-	-
TOTAL SEQUENCES		**0**	**1**	**0**	**1**	**0**	**0**	**0**	**1**	**0**	**0**
LDS	ArcPamv3B	√	-	√	-	-	-	-	-	-	-
TOTAL SEQUENCES		**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
		SS1	**SS2**								
TOTAL SEQUENCES		**53**	**62**								

SS1: Sample Station 1

SS2: Sample Station 2

Sp.: Spring

Su.: Summer

Au.: Autumn

Wi.: Winter