

Sediment bacteria and archaea community analysis and nutrient fluxes in a sub-tropical polymictic reservoir

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ABSTRACT: Lake sediments are important areas for remineralisation of nutrients involved in phytoplankton blooms. This study simultaneously analysed the microbial community structure and measured the sediment fluxes of inorganic nitrogen, phosphorus and silica from sediment cores collected at 2 different locations within a sub-tropical polymictic reservoir, Lake Wivenhoe. The bacterial and archaeal community structure was determined by amplifying and cloning the 16S rRNA gene from co-extracted DNA and RNA samples. A total of 19 phyla or candidate divisions of bacteria were identified, with sulphur-reducing bacteria within the phylum of *Deltaproteobacteria* being the most abundant ribotypes in DNA-derived clones libraries. In contrast, *Actinobacteria* and *Acidobacteria* were the most abundant ribotypes in RNA-derived clone libraries from the upper and lower sediments, respectively. The archaeal community was dominated by *Euryarchaeota*, with methanogenic archaea belonging to subdivisions of *Methanobacteria* and *Methanomicrobia* accounting for 69 to 98 % of the sequenced clones. Comparison of the 16S rDNA and rRNA clone libraries revealed that bacterial groups highly abundant in the sediments were mostly metabolically inactive, whilst those metabolically active were not very abundant. A higher relative abundance of nitrifying ribotypes (*Nitrospira* sp.) was identified at Site B, which corresponded to a higher efflux of nitrate from the sediments to the water column at this site. At the time of sampling, Lake Wivenhoe was stratified, and sediment cores were collected from the hypolimnion. Our results suggest that water column depth and delivery of dissolved oxygen to the sediments influenced the sediment microbial community structure and the fluxes and speciation of nutrients, which are reported to influence phytoplankton species composition and bloom dynamics.

KEY WORDS: Microbial community · Sediment · Nutrients · Bacteria · Archaea · 16S rRNA

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INTRODUCTION

Sediments in shallow water bodies are important areas of nutrient remineralisation and carbon processing and are also depositional zones. The large surface area generated by the sediment grains and strong gradients in organic substrates and terminal electron acceptors provide an ideal environment for exploitation

by many different microbial groups (Schwarz et al. 2007). Many of the microorganisms within the sediments play key roles in biogeochemical cycling of carbon, nitrogen, phosphorus and silica. In shallow lakes, these biogeochemical processes greatly influence phytoplankton blooms and water quality because of the intensive interchange of nutrients between the sediments and water column (Ye et al. 2009).

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Lake Wivenhoe is the largest water storage reservoir in south-eastern Queensland and supplies 80% of the drinking water of the city of Brisbane, Australia (Douglas et al. 2007). The lake is eutrophic and suffers from frequent blooms of toxic *Cyanobacteria* and periodic episodes of earthy odours (geosmin or 2-methylisoborneol), likely produced by either *Cyanobacteria* or *Actinomycetes* (Klausen et al. 2004, Zaitlin & Watson 2006). Research on phytoplankton bloom formation and sources of nutrients is currently underway in Lake Wivenhoe in an attempt to improve the quality of the water for drinking (Burford et al. 2012, Kerr et al. 2010, 2011a,b). Nitrogen and phosphorus are the 2 most important nutrients governing phytoplankton growth (Correll 1998). However, differences in the ratios of dissolved N:P:Si can change phytoplankton species composition within a lake because the growth of diatom species depends on the presence of dissolved silica, whereas the growth of non-diatom phytoplankton species (e.g. *Cyanobacteria*) does not (Conley et al. 1993).

Lake Wivenhoe is considered to be a polymictic lake because it experiences several periods of thermal stratification, separated by periods of overturn and fully mixed conditions, each year. During thermal stratification, the hypolimnion becomes anoxic from the biodegradation of organic material in the sediments by oxygen-consuming microorganisms. During anoxic conditions, ammonia is one of the first compounds to be released from the sediments as the biological oxidation of ammonia to nitrate (nitrification) is inhibited (Beutel et al. 2008), and the biological uptake of ammonia in anoxic conditions is relatively low compared to aerobic conditions (Beutel 2006). The sediments of lakes with oxic hypolimnia contain oxidized iron (Fe^{3+}) as $\text{Fe}(\text{PO}_4)$ and related hydroxides (Murray 1995). As the dissolved oxygen further decreases, Fe^{3+} is reduced, and Fe^{2+} and PO_4^{3-} are released into the water column (Murray 1995). Ammonia and phosphorus released into bottom waters from the sediments can exacerbate eutrophication when bottom waters mix with surface waters (Beutel et al. 2008). Ammonia is the preferred nitrogen source for the local toxic *Cyanobacteria*, *Cylindrospermopsis raciborskii* (Burford et al. 2006), and pulses of PO_4^{3-} were also shown to promote *C. raciborskii* dominance of the phytoplankton community (Posselt et al. 2009). Remineralisation rates of silica contained in diatom frustules within the sediments are largely controlled by physiochemical processes, such as the degree of under-saturation of dissolved silica in the water or the blocking of reactive sites by inhibitors, such as metal ions (Conley et al. 1993). However, prokaryotes can

enhance the dissolution of biogenic silica by removing organic coatings from diatom frustules and thereby enhancing the amount of 'clean' surface area exposed to under-saturated waters (Libes 2009).

The identification of the microorganisms within the sediments of Lake Wivenhoe is a key step towards understanding how N, P and Si are remineralised within this ecosystem. Thus, clone libraries of polymerase chain reaction (PCR)-amplified bacterial and archaeal 16S ribosomal genes from sediment samples were sequenced, and the potential biological and metabolic process of each microorganism within the clone library was inferred from the phylogenetic position of its sequence. Putative metabolic processes performed by sediment microbes were then compared to nutrient fluxes of dissolved N, P and Si. Because a large fraction of the microbial cells within the anoxic sediments may be dormant or dead (see Miskin et al. 1999), the relative abundances of ribotypes in clone libraries prepared from either DNA or RNA were compared to identify the active microbial fraction. Our results provide insight into biogeochemical processes occurring within the sediments of Lake Wivenhoe and the prokaryotes responsible for remineralisation of nutrients in the sediments.

MATERIALS AND METHODS

Study sites, sampling, core incubations and nutrient fluxes

Sediment cores were collected from 2 different locations (Site A, 27° 19.59 S, 152° 33.10 E, and Site B, 27° 20.40 S, 152° 32.46 E) within Lake Wivenhoe, SE Queensland, Australia. This slightly alkaline lake is shallow (the average depth is 10 m), with a surface area of 10 940 ha at full capacity (Douglas et al. 2007). The 2 sites chosen were known to differ in both physical (e.g. temperature and depth) and chemical (e.g. dissolved inorganic nitrogen and pH) parameters, based on routine water quality monitoring conducted on the lake by Seqwater. The physicochemical profile of each sampling site was determined at the time of sampling using a multi-parameter YSI 6600 V2 data sonde to measure dissolved oxygen, temperature and pH at 1 m increments. Three replicate sediment cores were collected from each site using a gravity corer fitted with acrylic liners (diameter 65 mm and length 400 mm), and 100 to 150 mm of sediment covered by ~300 mm of water was collected. Cores were capped with an airtight bung and transported to the laboratory (< 2 h), where they were

immediately placed in incubators filled with water from each site that had been passed through a 60 µm mesh screen. The incubator water was maintained at the measured ambient temperature (Site A $19 \pm 1^\circ\text{C}$ and Site B $20 \pm 1^\circ\text{C}$), and dark conditions were imposed because the sediment cores were collected from the aphotic zone. The water column within each core was gently mixed using a Teflon-coated magnetic stirrer positioned below the core cap at a rate that avoided sediment re-suspension. The cores were left overnight to settle, and incubations began when the dissolved oxygen returned to ambient levels (0.35 and 0.55 mg l⁻¹ at Site A and B, respectively). The sediment fluxes of dissolved inorganic nitrogen species (NH₄⁺, NO₂⁻ and NO₃⁻), filterable reactive phosphorus (FRP), total phosphorus (TP) and reactive silica were estimated by withdrawing water using a plastic syringe at 6 h intervals over a 24 h period from the sample port located in the incubator core cap. Water samples were filtered (0.45 µm), frozen at -20°C and subsequently assayed for the above parameters colourimetrically using a flow injector analyser by Queensland Health, Forensic & Scientific Services. Dissolved methane fluxes were estimated by withdrawing 6 ml of each water sample from the sample port and analysing the methane content using a gas chromatograph as described by O'Sullivan et al. (2005). Fluxes of methane, silica, nitrogen and phosphorus species across the sediment-water interface were calculated by linear regression of the concentration data as a function of time, core water volume and surface area (Eyre & Ferguson 2002).

At the end of the incubations, sediment samples from the upper (0 to 50 mm) and lower sediments (100 to 150 mm) were taken using a sterile serological pipette from one core per site. Sediment samples were immediately frozen in liquid nitrogen and stored at -80°C for subsequent nucleic acid extraction.

Co-extraction of RNA/DNA and PCR amplification

Total genomic DNA and total RNA were co-extracted from sediment core samples using the RNA PowerSoil™ Total RNA Isolation kit and the DNA Elution Accessory kit (Mo Bio Laboratories). The purified total RNA was DNase treated using the RNeasy mini kit with the RNase-Free DNase set (Qiagen), and 200 ng of total RNA per sample was reverse-transcribed using the Superscript® III First-Strand Synthesis System (Invitrogen). Community ribosomal DNAs or cDNAs were PCR amplified in 3

parallel 25 µl PCR reactions using Platinum® *Taq* DNA Polymerase High Fidelity (Invitrogen) and 300 nM of each of the universal bacterial primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GTT ACC TTG TTA CGA CTT-3') or universal archaeal primers 109aF (5'-ACK GCT CAG TAA CAC GT-3') and 1119aR (5'-GGY RSG GGT CTC GCT CGT T-3'). Replicate amplification products were pooled to account for PCR bias, purified using a NucleoSpin® Extract II kit (Macherey-Nagel) and cloned using the pCR4® TOPO cloning kit (Invitrogen). The PCR inserts from 95 positive clones from each library were amplified using PCR and forward sequenced using gene specific primers (27F or 109aF) by the Australian Genome Research Facility. High quality partial sequences were aligned using ClustalW to allow the identification of identical or nearly identical sequences, and 114 bacterial and 92 archaeal clones were selected for complete sequencing using M13F and M13R primers as above.

Phylogenetic analysis

The obtained sequences were quality scored and visually checked for possible sequencing errors before compilation using Sequencher version 4.9 (Gene Codes Corporation). The sequences were then compared to the available databases using the basic local alignment search tool (BLAST) network service (Altschul et al. 1997) to determine their approximate phylogenetic affiliations. Chimeric sequences were identified and removed from further analysis using Bellerophon (Huber et al. 2004) and Mallard (Ashelford et al. 2006). Sequences were then aligned with selected reference 16S rRNA sequences from GenBank using the ClustalW algorithm in Mega version 4.0 (Tamura et al. 2007), and phylogenetic trees were constructed using the neighbour-joining distance method (Saitou & Nei 1987). The statistical significance of interior nodes was determined by performing bootstrap analyses based on 1000 re-samplings of the data. Sequences described in this study were submitted to GenBank and assigned accession numbers from HQ330530 to HQ330736.

Identification of ammonia-oxidising archaea and bacteria

The alpha-subunit of the ammonia monooxygenase (*amoA*) gene was PCR amplified from genomic DNA and cDNA in an attempt to determine the bac-

teria and archaea responsible for ammonia oxidation within the sediments. The archaeal *amoA* gene was amplified using the primers Arch-*amoA*F (5'-STA ATG GTC TGG CTT AGA CG-3') and Arch-*amoA*R (5'-GCG GCC ATC CAT CTG TAT GT-3') following the PCR conditions described by Francis et al. (2005). The bacterial *amoA* gene was amplified using the primers *amoA*-1F (5'-GGG GTT TCT ACT GGT GGT-3') and *amoA*-2R (5'-CCC CTC KGS AAA GCC TTC TTC-3') following similar PCR conditions described by Rotthauwe et al. (1997). Duplicate PCR products were pooled, purified (QIAquick PCR Purification kit, Qiagen) and visualised on a 1% agarose/ethidium bromide gel alongside a 1 kb DNA ladder (Fermentas).

RESULTS

Water column profile and sediment flux of nutrients

Strong gradients in water temperature, dissolved oxygen concentration and pH were observed at both Sites A and B (Fig. 1). The depth at Site A and B was 29 and 15 m, respectively. The concentration of dissolved oxygen, pH and temperature was measured directly (0.5 m) above the water/sediment interface, and these parameters were found to be marginally lower at Site A (Fig. 1).

The daily flux of nutrients from sediment cores is presented in Fig. 2. A greater efflux of silica was observed from sediment cores collected from Site A

(Fig. 2). Ammonia fluxes differed between sampling sites, with a strong efflux of ammonia observed in cores collected from Site A, whilst a slight influx of ammonia was observed in sediment cores from Site B (Fig. 2). Patterns of nitrate and nitrite fluxes were similar between sites; however, the flux rates differed greatly, with the nitrate efflux almost 3-fold greater at Site B, whilst the nitrite influx was over 5-fold lower. The concentration of NH_4^+ , NO_3^- and FRP measured in the bottom waters was in accordance with differences in flux rates from sediment cores collected from Sites A and B (data not shown). A strong efflux of methane was also measured from sediment cores (Fig. 3), with the methane flux almost 4-fold greater from sediment cores collected from Site A.

Bacterial community analysis

A total of 570 bacterial clones were randomly selected for sequencing from 4 bacterial 16S rDNA and 2 bacterial 16S rRNA clone libraries (95 clones per library), which resulted in 476 high quality partial sequences for further analysis. These partial sequences were clustered into 114 groups, and representatives from each group were forward- and reverse-sequenced, yielding 87 identified operational taxonomic units (OTUs), based on a 97% cut-off. A total of 14 chimeric sequences were detected and deleted from analysis. The number of clones analysed per library is presented in Table 1. Rarefaction analysis of the 6 samples (Fig. 4) indicated that sampling did not reach a diversity asymptote based on 97% similarity clustering. Less than 40% of the 16S rRNA gene sequences in the current study were affiliated with already existing sequences in the public database with 97% identity (see Table S1 in the supplement at www.int-res.com/articles/suppl/a065p287_supp.pdf). These 16S rRNA gene sequences had high similarities to those of uncultured environmental prokaryotes retrieved from a phreatic sinkhole (Sahl et al. 2010), wetlands (D'Auria et al. 2010) and lake (Bri e et al. 2007, Schwarz et al. 2007, Ye et al. 2009) and estuarine sediment (Jiang et al. 2009). The remaining sequences were remotely related to 16S rRNA gene sequences of known bacteria and represent novel phylotypes not described in previous studies.

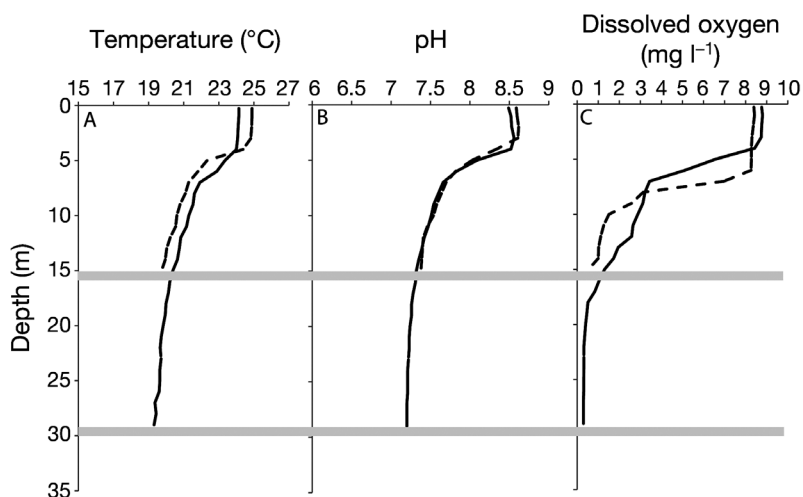


Fig. 1. Physiochemical profile of the water column measured at 1.0 m increments prior to core collection at Site A (continuous line) and Site B (dashed line). Sediment depths (thick horizontal grey lines) were 29 m (Site A) and 15 m (Site B)

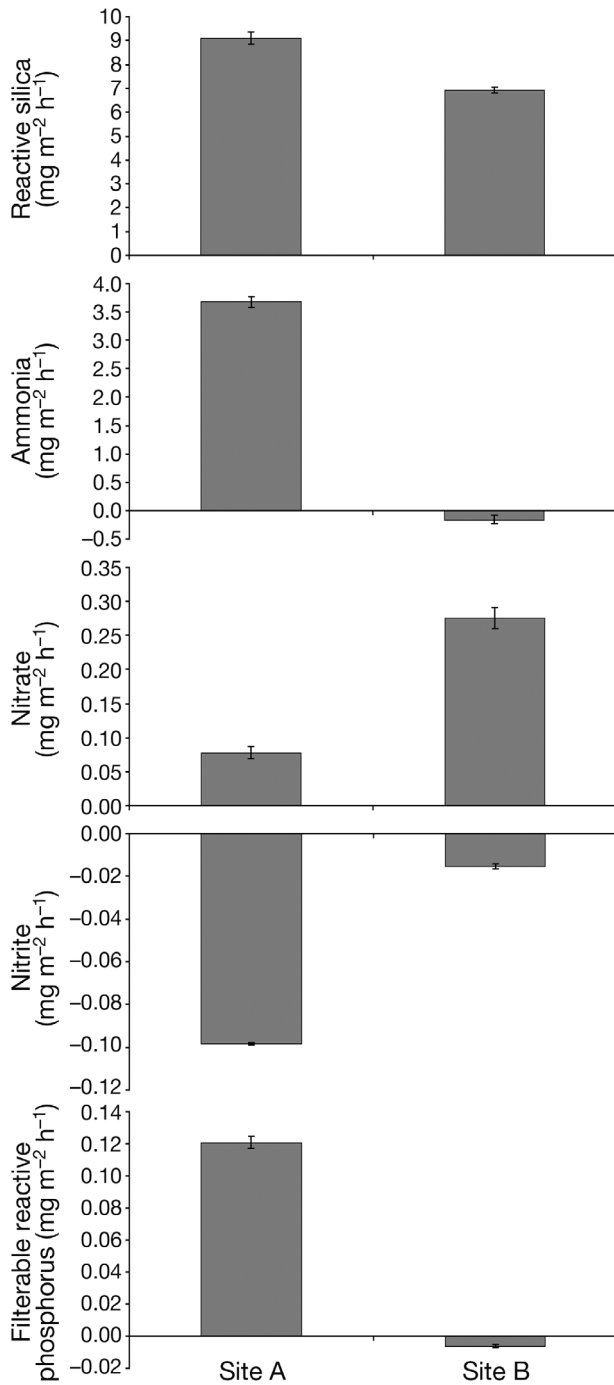


Fig. 2. Sediment nutrient fluxes of dissolved inorganic silica, nitrogen and phosphorus species from sediment cores collected at Sites A and B. Means \pm SE are shown

Phylogenetic analysis (Figs. 5, 6 & 7) indicated that the bacterial community of the sediments within Lake Wivenhoe consisted of *Alpha*-, *Beta*-, *Delta*- and *Gammaproteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chlorobi*, *Chloroflexi*,

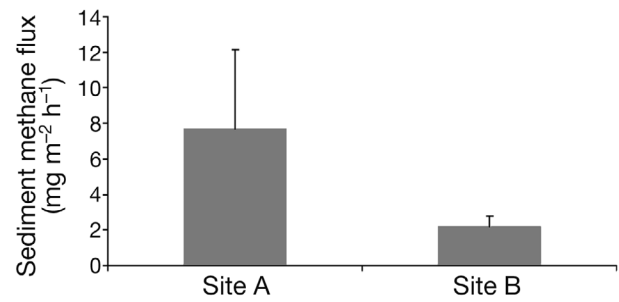


Fig. 3. Methane flux rates from sediment cores collected at Sites A and B. Means \pm SE are shown

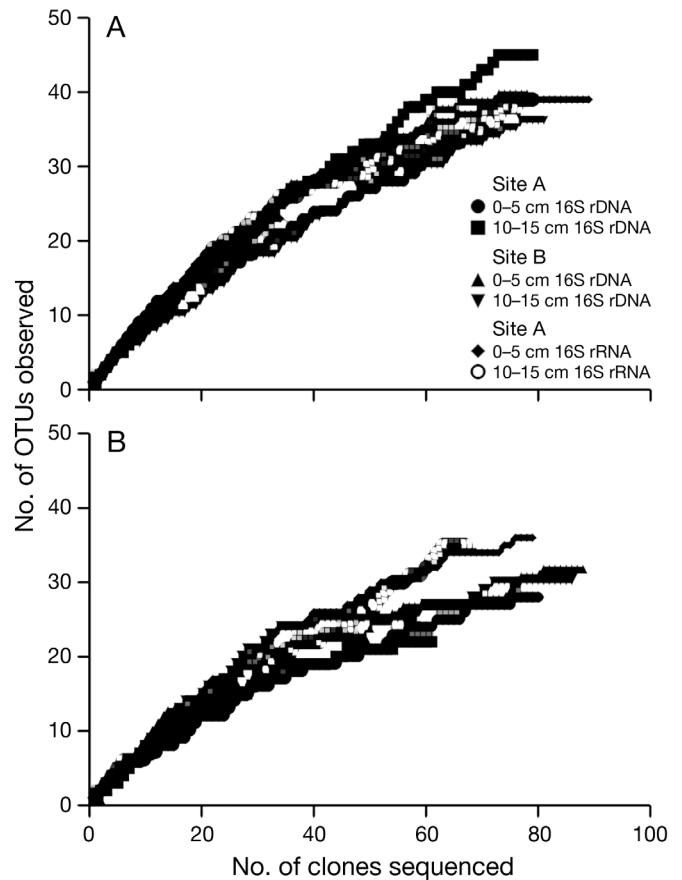


Fig. 4. Rarefaction curves based on 97% similarity of (A) bacterial and (B) archaeal clone libraries. Libraries were generated from either genomic DNA (16S rDNA) or total RNA (16S rRNA) at Site A, whereas libraries at Site B were only generated from genomic DNA. OTU: operational taxonomic unit

Cyanobacteria, *Deinococcus-Thermus*, *Firmicutes*, *Nitrospira*, *Planctomycetes*, *Spirochaetes*, *Verrucomicrobia* and the candidate divisions WS3, TM6, OP1, OP3, OP8 and OP11. The diversity (Shannon's index), number of clones analysed, relative abun-

Table 1. Distribution of 16S rRNA bacterial ribotypes within each phylum in DNA- and RNA-based analyses between upper (0–50 mm) and lower (100–150 mm) sediments at Sites A and B. The relative abundance (%) of clones for each phylum within a particular sample is based on forward partial sequences. The Shannon diversity index (H') and the number of high quality partial sequences analysed (n) from each library is also presented. rDNA (rRNA): ribotypes from sediment-extracted DNA (RNA or cDNA) libraries

Phylum	Class	Order	Site A				Site B	
			0–50 mm		100–150 mm		0–50 mm	100–150 mm
			rDNA	rRNA	rDNA	rRNA	rDNA	rDNA
<i>Proteobacteria</i>	<i>Alpha</i>		0.0	0.0	0.0	0.0	2.4	1.4
<i>Proteobacteria</i>	<i>Beta</i>	<i>Burkholderiales</i>	0.0	0.0	3.6	0.0	3.7	2.7
<i>Proteobacteria</i>	<i>Beta</i>	<i>Methylophilales</i>	5.2	0.0	0.0	0.0	1.2	2.7
<i>Proteobacteria</i>	<i>Beta</i>	Unclassified	0.0	0.0	3.6	1.3	2.4	2.7
<i>Proteobacteria</i>	<i>Delta</i>	<i>Desulfobacterales</i>	13.0	3.4	18.1	1.3	9.8	13.5
<i>Proteobacteria</i>	<i>Delta</i>	<i>Desulfuromonadales</i>	2.6	1.1	1.2	0.0	1.2	4.1
<i>Proteobacteria</i>	<i>Delta</i>	<i>Myxococcales</i>	0.0	2.2	1.2	0.0	1.2	0.0
<i>Proteobacteria</i>	<i>Delta</i>	<i>Syntrophobacterales</i>	11.7	4.5	8.4	6.5	6.1	6.8
<i>Proteobacteria</i>	<i>Delta</i>	Unclassified	2.6	1.1	10.8	2.6	7.3	17.6
<i>Proteobacteria</i>	<i>Gamma</i>	<i>Methylococcales</i>	11.7	6.7	7.2	1.3	7.3	5.4
<i>Proteobacteria</i>	<i>Gamma</i>	<i>Pseudomonadales</i>	0.0	0.0	0.0	5.2	0.0	1.4
<i>Proteobacteria</i>	<i>Gamma</i>	<i>Xanthomonadales</i>	2.6	2.2	0.0	0.0	0.0	2.7
<i>Proteobacteria</i>	<i>Gamma</i>	<i>Legionellales</i>	0.0	0.0	0.0	0.0	0.0	1.4
<i>Verrucomicrobia</i>			5.2	3.4	3.6	1.3	9.8	4.1
<i>Acidobacteria</i>			7.8	9.0	9.6	20.8	7.3	4.1
<i>Actinobacteria</i>			1.3	20.2	2.4	6.5	1.2	0.0
<i>Firmicutes</i>			5.2	1.1	0.0	1.3	0.0	0.0
<i>Chloroflexi</i>			3.9	11.2	1.2	7.8	2.4	0.0
<i>Cyanobacteria</i>			0.0	2.2	0.0	5.2	1.2	0.0
<i>Nitrospira</i>			0.0	0.0	3.6	1.3	8.5	2.7
<i>Spirochaetes</i>			1.3	0.0	1.2	5.2	1.2	0.0
<i>Planctomycetes</i>			3.9	3.4	2.4	1.3	1.2	8.1
<i>Deinococcus-Thermus</i>			1.3	0.0	0.0	0.0	0.0	0.0
<i>Chlorobi</i>			1.3	0.0	0.0	0.0	0.0	2.7
<i>Bacteroidetes</i>			15.6	1.1	9.6	1.3	11.0	9.5
OP1			0.0	4.5	0.0	13.0	0.0	0.0
OP8			1.3	2.2	1.2	3.9	0.0	0.0
OP11			2.6	6.7	2.4	2.6	2.4	1.4
TM6			0.0	2.2	3.6	0.0	4.9	1.4
WS3			0.0	4.5	1.2	2.6	0.0	1.4
Unknown			0.0	4.7	3.6	3.9	6.1	2.8
H'			3.2	3.4	3.5	3.4	3.5	3.3
n =			82	92	87	87	86	87

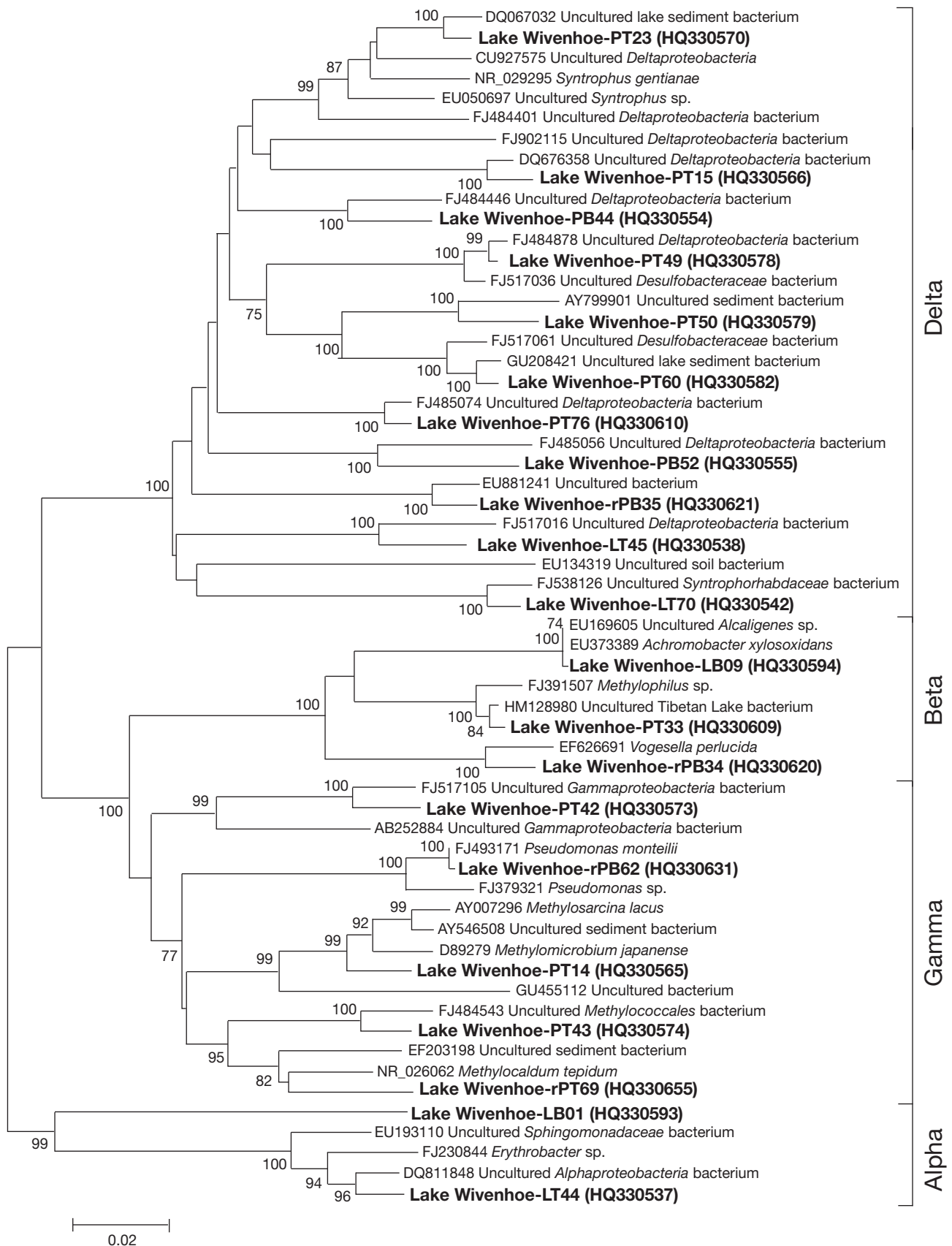
dance and frequency of clones within each major phylogenetic division for each clone library are presented in Table 1.

Archaeal community analysis

A total of 570 clones from archaeal 16S rDNA and rRNA clone libraries were randomly sequenced, and 458 high quality partial sequences were obtained and grouped into 74 clusters. Representatives from each cluster were forward- and reverse-sequenced, and 51 OTUs were identified, based on a 97% cut-off. A total of 9 chimeras were detected and removed from the phylogenetic analysis. Almost 70% of the

16S rRNA gene sequences identified in the current study were affiliated with already existing sequences in the public databases with 97% identity (see Table S2 in the supplement at www.int-res.com/articles/suppl/a065p287_supp.pdf). These 16S rRNA

Fig. 5. Unrooted phylogenetic tree of proteobacterial 16S rRNA sequences from the current study and related reference sequences. Sequences from the current study are designated as Lake Wivenhoe, followed by the clone ID and GenBank accession number in brackets. The tree was constructed using the neighbour-joining method using nearly full-length aligned nucleotide sequences in MEGA version 4. Bootstrap values (%) are based on 1000 replicates and are shown at the nodes with >70% support. Scale bar represents 2% sequence divergence



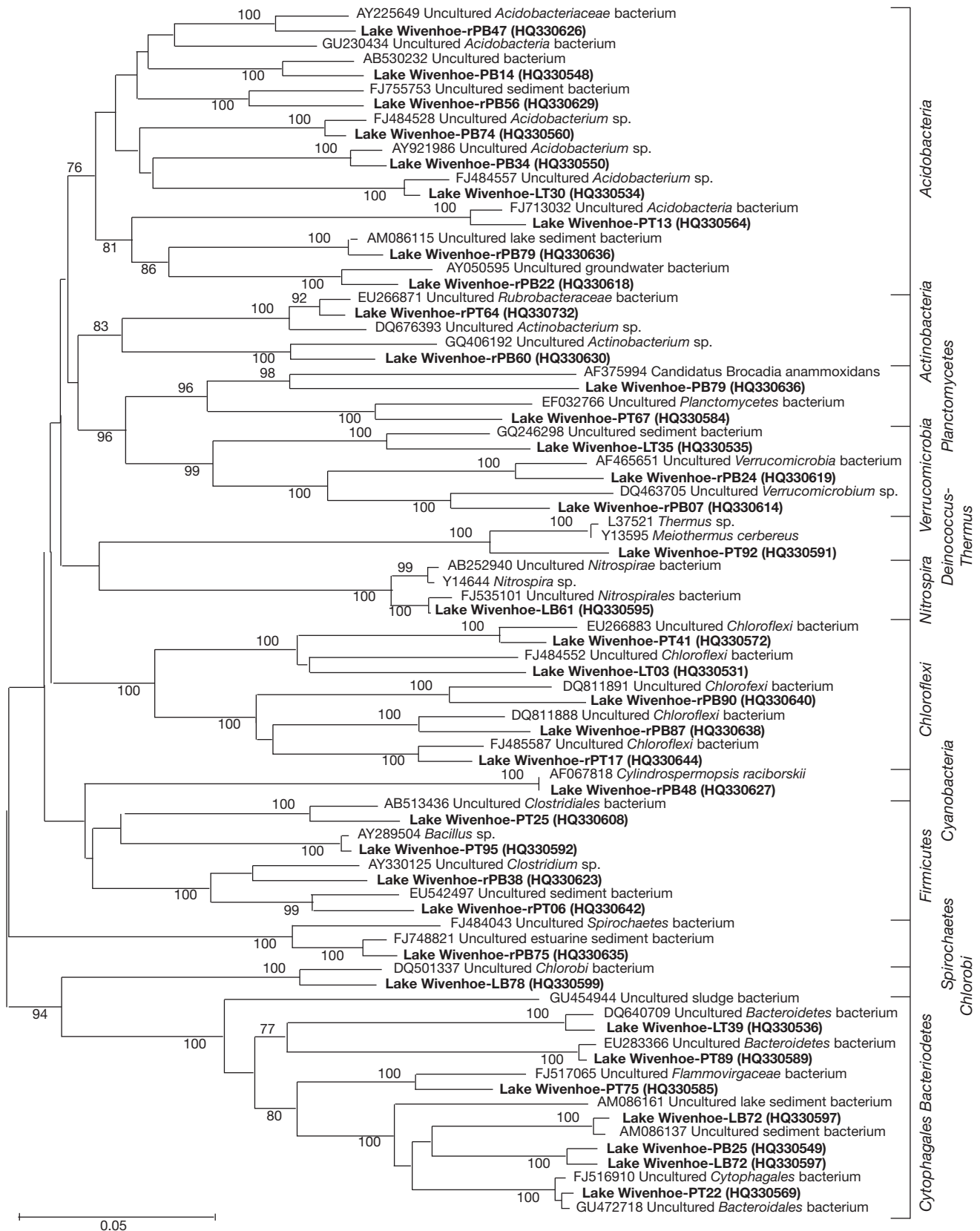


Fig. 6. Unrooted phylogenetic tree of classified bacterial 16S rRNA sequences, except those of *Proteobacteria*. For details see Fig. 5 legend. Scale bar represents 5% sequence divergence

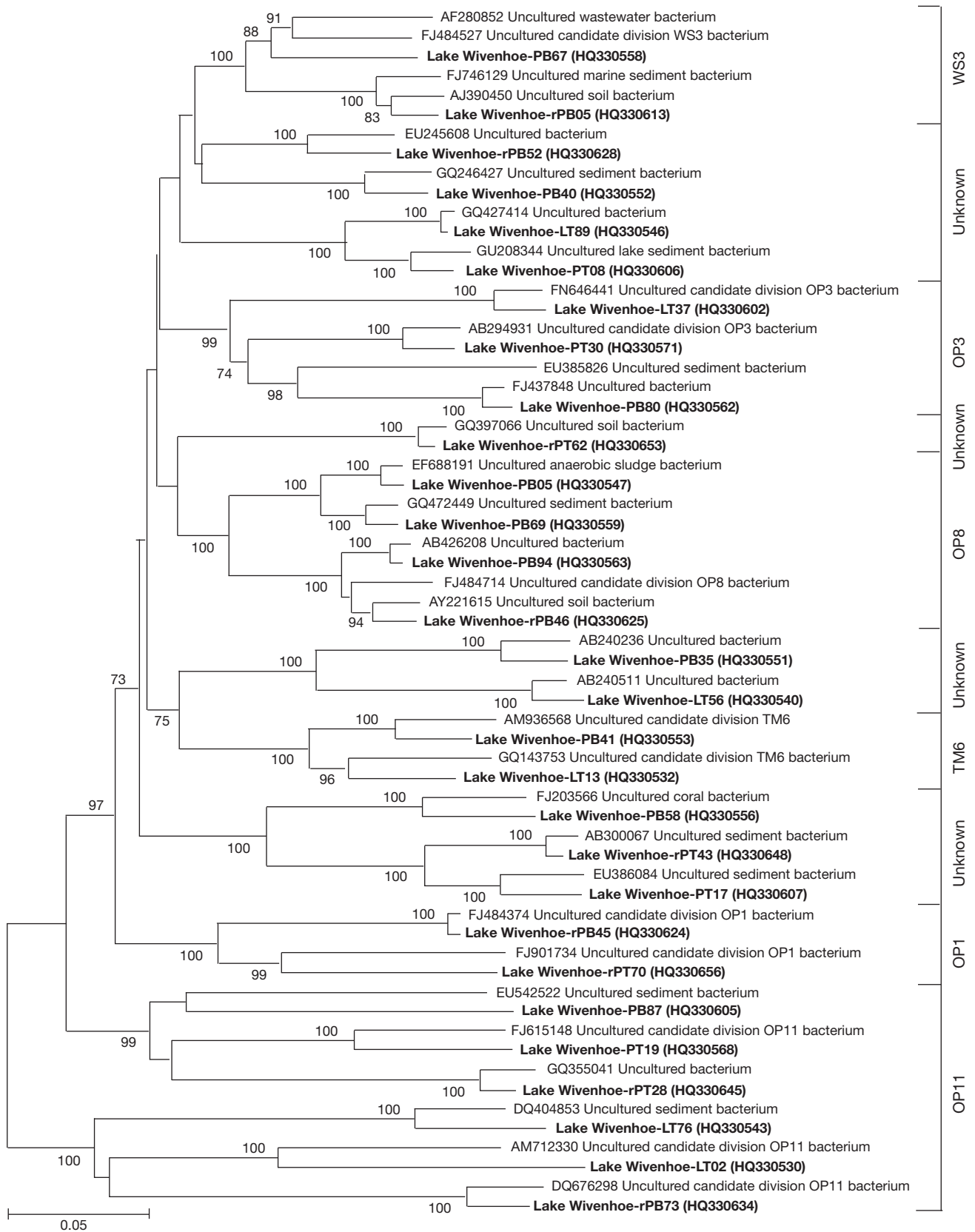


Fig. 7. Unrooted phylogenetic tree of unclassified bacterial 16S rRNA sequences retrieved in the current study. For details see Fig. 5 legend. Scale bar represents 5% sequence divergence

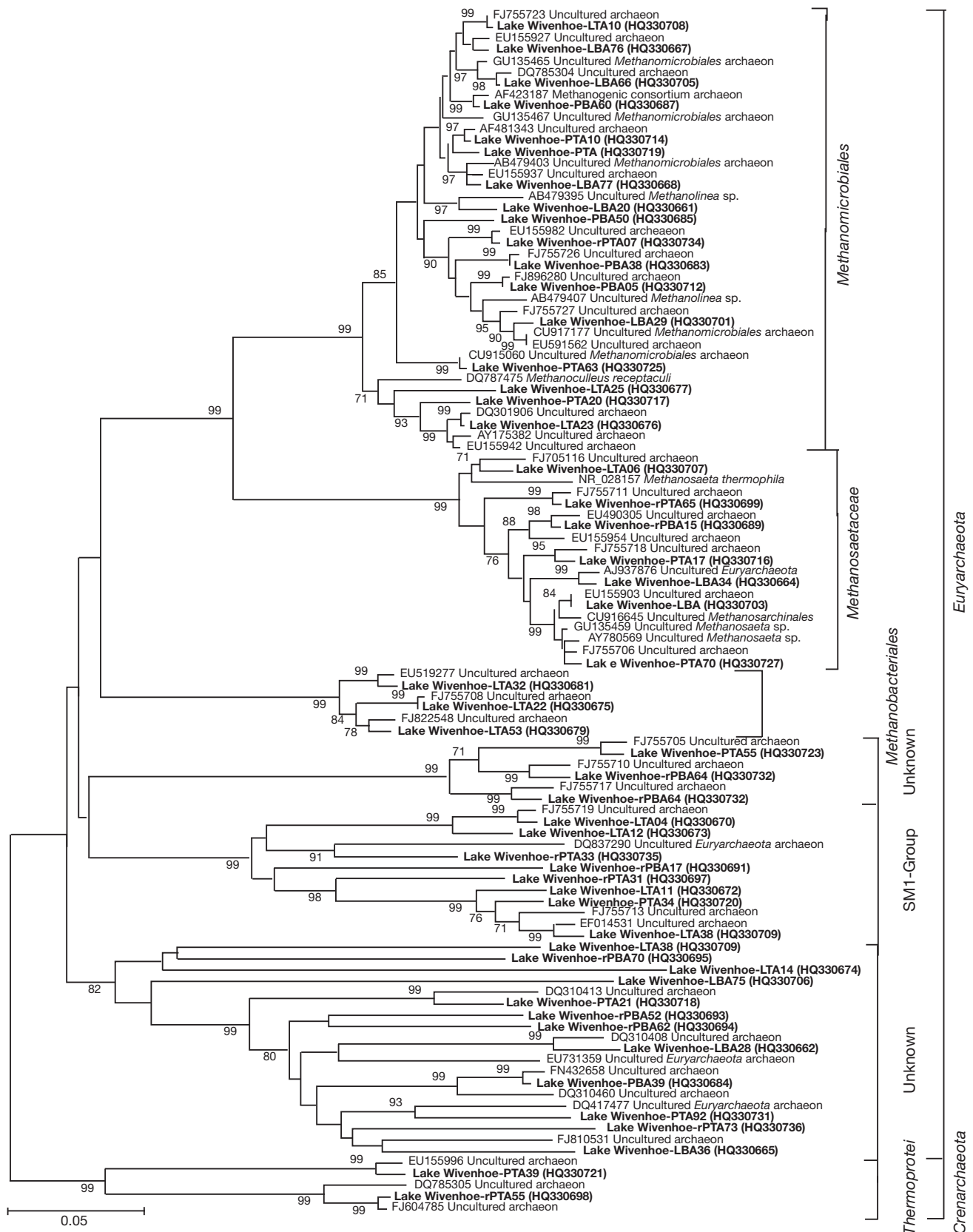


Fig. 8. Unrooted phylogenetic tree of archaeal 16S rRNA gene sequences retrieved in the current study and related reference sequences. For details see Fig. 5 legend. The scale bar represents 5% sequence divergence

gene sequences had high similarities to those of uncultured methanogenic archaea (Fig. 8) retrieved from freshwater lake sediments (Lehours et al. 2007, Ye et al. 2009), wastewater sludge (Rivière et al. 2009), minerotrophic fen (Cadillo-Quiroz et al. 2008), acidic bogs (Chan et al. 2002) and peatlands (Basiliko et al. 2003, Cadillo-Quiroz et al. 2006). The remaining 24 sequences were remotely related to 16S rRNA gene sequences of known archaea and represent novel phylotypes not described in previous studies (see Table S2 in the supplement). The majority of 16S rRNA gene sequences in the current study belonged to either *Methanomicrobiales* or *Methanosaeta*, and the diversity, number of clones analysed and relative abundance of the major phylogenetic divisions in each archaeal 16S rDNA or rRNA clone library is presented in Table 2.

Ammonia-oxidising archaea and bacteria

Numerous attempts to amplify the archaeal and bacterial *amoA* gene from genomic DNA and cDNA isolated from Lake Wivenhoe sediment samples were unsuccessful. In each case, positive controls were successfully amplified. Additions of PCR enhancers (3 to 5% dimethyl sulfoxide in master mix) and modification of PCR parameters, such as annealing temperatures and number of cycles, were also unsuccessful for amplifying the *amoA* gene from Lake Wivenhoe samples.

DISCUSSION

The sediments of Lake Wivenhoe presumably contain organic material from the likely accumula-

tion of autochthonous material (e.g. deceased phytoplankton) from the above surface waters. This organic material is utilised by a diverse microbial community for energy and cellular carbon and in the process is remineralised back into the water column. Although the temperature, pH and dissolved oxygen concentrations measured directly (0.5 m) above the water/sediment interface at the 2 study sites were only marginally different (Fig. 1), water column depth clearly influenced both nutrient flux rates (Fig. 2) and microbial community structure (Tables 1 & 2). At Site A, the measured dissolved oxygen penetrated to a water depth of 18 m, which is approximately 11 m above the water/sediment interface. In contrast, Site B is shallower (15 m), and diffused dissolved oxygen would reach the water/sediment interface and be rapidly consumed by the sediment microbial community (Fig. 1). This supply of dissolved oxygen to the sediments at Site B would alter the metabolism and structure of the sediment microbial community. The following discussion speculates on the biogeochemical processes performed by the different members of bacteria and archaea observed in the sediments cores collected from the 2 different locations and how these microbial communities influence the remineralisation and flux of nutrients known to be involved in phytoplankton blooms.

Microbial diversity

The current study identified a total of 87 bacteria and 57 archaea in the upper and lower sediments of Lake Wivenhoe. However, the true bacterial and archaeal diversity within the sediments is still likely to be underestimated, as indicated by rarefaction

Table 2. Distribution of 16S rRNA archaea ribotypes within each phylum in DNA- and RNA-based analyses between upper and lower sediments at Sites A and B. The relative abundance of clones for each group within a particular sample is presented as a percentage. Shannon diversity index (H') and the number of high quality partial sequences analysed (n) from each library is also presented. rDNA (rRNA): ribotypes from sediment-extracted DNA (RNA or cDNA) libraries

Phylum	Class	Order	Site A				Site B	
			0–50 mm		100–150 mm		0–50 mm	100–150 mm
			rDNA	rRNA	rDNA	rRNA	rDNA	rDNA
<i>Crenarchaeota</i>	<i>Thermoprotei</i>		1.1	2.1	0.0	1.1	0.0	0.0
<i>Euryarchaeota</i>	<i>Methanobacteria</i>	<i>Methanobacteriales</i>	2.2	2.1	1.1	0.0	3.2	4.3
<i>Euryarchaeota</i>	<i>Methanomicrobia</i>	<i>Methanomicrobiales</i>	53.8	48.9	69.0	57.8	50.5	49.5
<i>Euryarchaeota</i>	<i>Methanomicrobia</i>	<i>Methanosarcinales</i>	35.2	26.6	27.6	21.1	34.7	41.9
Unknown			7.7	20.2	2.3	20.0	11.6	4.3
H'			2.9	3.2	2.8	3.2	3.1	3.1
n			91	94	87	90	95	93

curves, which do not reach a diversity plateau (Fig. 4). Despite this, the bacterial and archaeal diversity (based on the Shannon index) was high and ranged from 3.2 to 3.5 and 2.8 to 3.2, respectively, in the sediments of Lake Wivenhoe (Tables 1 & 2). The obtained diversity values in the current study are similar to those of other studies investigating sediment communities using similar techniques (Bri e et al. 2007, Schwarz et al. 2007).

Role of microbes in biogeochemical cycling

Dissolved reactive silica fluxes were greater from sediment cores collected at Site A (Fig. 2), which was supported by a significantly higher silica concentration in the bottom waters at this site (Fig. S2 in the supplement at www.int-res.com/articles/suppl/a065p287_supp.pdf). The sediments of Lake Wivenhoe presumably contain biogenic silica from the sedimentation of deceased diatoms. Remineralisation of biogenic silica is largely controlled by abiotic factors (Conley et al. 1993), but microbes can enhance the dissolution of biogenic silica by removing organic coatings from diatom frustules (Libes 2009). Degradation of organic matter within the sediments requires multiple steps performed by different trophic levels of bacteria and archaea. Heterotrophs are responsible for the initial microbial degradation of complex organic material within the sediments and therefore fill the lowest trophic level. These heterotrophs utilize this complex organic material for energy and cellular carbon and excrete a range of simple organic (e.g. acetate) and inorganic (e.g. urea and ammonia) molecules, which are then further catabolised by higher trophic levels of chemolithotrophs. Acetate can be assimilated into cellular carbon and further metabolised to methane and CO₂ by acetoclastic methanogenesis or completely oxidised to CO₂ by sulphate-reducing bacteria (Winfrey & Zeikus 1977).

Actinobacteria and *Acidobacteria* were likely to be the most metabolically active heterotrophic bacteria within the upper and lower sediments, respectively, of Lake Wivenhoe based on the frequency of 16S rRNA ribotypes in clone libraries. Other heterotrophs, including *Verrucomicrobia*, *Firmicutes* and *Pseudomonas* sp., were also identified in the 16S rDNA libraries and hence may also participate in the degradation of organic material. *Actinobacteria* constitute a diverse phylum, with members possessing highly variable physiological and metabolic properties, and are abundant in environmental soil

and sediment samples (Ventura et al. 2007). *Actinobacteria* play an important role in carbon cycling and have been shown to excrete a wide range of enzymes that degrade complex organic carbons (lignin) during composting of agricultural waste (Yu et al. 2007). *Actinobacteria* in the upper sediments of Lake Wivenhoe may play an important role in removing the complex polysaccharide coatings from diatom frustules and exposing biogenic silica to under-saturated waters.

Sulphate reduction is thought to outcompete methanogenesis when sulphate is freely available because cultured strains of sulphate-reducing bacteria have higher specific affinities than methanogenic bacteria and archaea for the main substrates (acetate and H₂) used by both groups (Purdy et al. 2003). However, available sulphate is usually limiting in freshwater environments, resulting in the dominance of methanogenesis as the main microbial pathway for organic carbon degradation in freshwater systems (Purdy et al. 2003). The high relative abundance of both sulphate-reducing bacteria and methanogenic archaea within the 16S rRNA clone libraries suggests that both of these microbial pathways do occur in the sediments of Lake Wivenhoe (Table 1). Sulphate-reducing bacteria (*Desulfobacterales*, *Desulfuromonadales* and *Syntrophobacterales*) were the most frequently observed ribotypes in 16S rDNA clone libraries from Sites A and B (Table 1). However, sulphate-reducing bacteria were likely to be metabolically inactive at the time of sampling based on the ratio of 16S rRNA to 16S rDNA sequences observed (Table 1). Sulphate reduction and methanogenesis can occur simultaneously in anoxic, sulphate-containing sediments if methanogens utilise substrates (e.g. methanol) not required by sulphate reducers (Oremland & Polcin 1982), or production of methane occurs from methanogenic CO₂ reduction by syntrophic consortia of acetate-oxidising bacteria and H₂/CO₂-using methanogens (N usslein et al. 2001). The majority of archaea identified in the present study had 16S rRNA sequences related to *Methanomicrobiales* (49 to 69%), *Methanosaeta* (21 to 41%) and *Methanobacterium* (2 to 4%), suggesting that methane is produced in the sediments of Lake Wivenhoe by both methanogenic H₂/CO₂ reduction (via *Methanomicrobiales* and *Methanobacterium*) and acetoclastic methanogenesis (via *Methanosaeta*) (Chan et al. 2002). Complete degradation of organic carbon in freshwater sediments by a syntrophic partnership of hydrogenotrophic and acetoclastic methanogens was recently suggested by

Biderre-Petit et al. (2011) and also appears to be the case in Lake Wivenhoe.

The measured flux of methane from the sediments of Site A was almost 4-fold greater than at Site B (Fig. 3). Increased methane production at Site A may be the result of a higher abundance or metabolic rate of methanogenic archaea, but methane is more likely being oxidised at the water–sediment interface at Site B. In freshwater environments, methane is usually oxidised at the oxic/anoxic interface by aerobic bacteria (Segers 1998). The diffusion of dissolved oxygen to the water/sediment interface at Site B presumably allows aerobic methane oxidation to occur by *Methylococcales* ribotypes (Type I methanotrophs), which were observed in 16S rRNA clone libraries. Methane can also be oxidised by anaerobic methane-oxidising archaea (ANME), which have been identified from the anoxic water body of the eutrophic freshwater Lake Plußsee (Eller et al. 2005). In the marine environment, anaerobic methane oxidation is reportedly coupled to sulphate reduction (Thomsen et al. 2001). No evidence exists to suggest this pathway occurs in freshwater systems (Knittel & Boetius 2009), and anaerobic methane oxidation in freshwater environments may instead be coupled with reduction of nitrite to dinitrogen (Ettwig et al. 2010). No 16S rRNA sequences related to ANME were retrieved in the current study.

The efflux of FRP was greater from the cores collected from Site A. Studies have shown that PO_4^{3-} cycling in freshwater lakes is regulated by the availability of and complexation with oxidised iron (Kleeberg 1997). The sediments of lakes with oxic hypolimnia contain oxidised iron (Fe^{3+}), as FePO_4 , and related hydroxides (Murray 1995). As thermal stratification occurs, the hypolimnion becomes anoxic, and the reductive dissolution of Fe^{3+} releases ferrous Fe^{2+} and PO_4^{3-} into the water column (Murray 1995). Our study identified a wide range of microorganisms in the sediments of Lake Wivenhoe that are capable of reducing Fe^{3+} for energy and growth, including members within sulphur-reducing bacteria, methanogenic archaea, *Acidobacteria* and *Deinococcus-Thermus* (Lovley et al. 2004). The greater efflux of FRP at Site A is most likely the result of the fully anoxic conditions at this site allowing a higher abundance and/or metabolic activity of Fe^{3+} reducers.

Decomposition of phytoplankton in the sediments generates ammonia primarily from the deamination of amino acids and urea (Jones et al. 1982). Ammonia is converted to nitrate in a 2-step process termed 'nitrification', which is performed by 2 distinct groups

of aerobic chemolithoautotrophic microbes. One step oxidises ammonia to nitrite (conducted by ammonia-oxidising bacteria or archaea), whereas another group oxidises nitrite to nitrate (Arp & Stein 2003). The fully anoxic conditions at Site A did not support nitrification, and >97% of the nitrogen efflux at this site was ammonia. In contrast, the supply of dissolved oxygen to the water/sediment interface at Site B supported nitrification, with 100% of the nitrogen efflux as nitrate (Fig. 2). The frequency of *Nitrospira* ribotypes (aerobic nitrite oxidisers) was also highest in the upper sediment clone library from Site B, suggesting that *Nitrospira* ribotypes play an important role in nitrite oxidation in Lake Wivenhoe. However, the microbes responsible for ammonia oxidation at Site B could not be determined in the current study. Most ammonia-oxidising bacteria (AOB) fall within the *Nitrosomonas* order of the *Betaproteobacteria* (Arp & Stein 2003), which were not detected in the 16S rDNA libraries in the present study. Recently, ammonia-oxidising archaea (AOA) were discovered and have been identified in the water column and sediments of freshwater lakes (Pouliot et al. 2009, Ye et al. 2009). These AOA belong to the *Crenarchaeota* phylum and are known to occur within the suboxic zone (<0.32 mg l⁻¹ of dissolved oxygen) (Coolen et al. 2007). Attempts to identify AOA and AOB by amplifying the *amoA* gene were unsuccessful, suggesting that ammonia oxidation is possibly performed instead by ammonia-oxidising heterotrophs (e.g. *Alcaligenes* spp.) or methane oxidisers (e.g. *Methylococcales*), and further research is warranted to determine if these heterotrophs are responsible for ammonia oxidation in the sediments of Lake Wivenhoe.

Ammonia could also be oxidised anaerobically with nitrite to dinitrogen (anammox) within the sediments of Lake Wivenhoe by bacteria falling in the order of *Planctomycetales* (Jetten et al. 2003). Planctomycete phylotypes related to environmental sequences were identified in the present study, with their highest relative abundance in the lower sediments of Site B. However, because *Planctomycetales* are physiologically diverse, it is not possible to comment on whether the planctomycete phylotypes identified in the sediments of Lake Wivenhoe are indeed involved in anammox. Theoretically, anaerobic ammonia oxidation can also be coupled to manganese or iron reduction to produce nitrite or dinitrogen (Feammox) (Hulth et al. 1999), but this pathway is yet to be quantified in nature. Nitrite production has been observed under Fe-reducing conditions in wetland sediments, suggesting that Feammox could exist in sediments (reviewed by Burgin et al. 2011).

CONCLUSIONS

Our study has contributed to the overall understanding of the role that microorganisms play in nutrient remineralisation in freshwater sediments. Although the type of analysis carried out here cannot quantify the abundance of bacteria and archaea in the sediments of Lake Wivenhoe, it constitutes the basis for assessing the relative importance of specific groups in nutrient remineralisation and carbon processing. Our results demonstrated that physicochemical properties of the water column (depth of water column and diffusion of dissolved oxygen) influenced the sediment microbial community structure. Furthermore, our study showed that the bacteria present in the sediments (based on 16S rDNA ribotypes) were not necessarily metabolically active (based on 16S rRNA ribotypes) at the time of sampling. Differences in the observed sediment flux rates and speciation of nutrients between the 2 study sites was likely the result of differences in the metabolic processes performed by the corresponding microbial communities. Differences in the flux and speciation of these nutrients released from the sediments into the hypolimnion could influence phytoplankton species composition and bloom dynamics when the hypolimnion mixes with the epilimnion during overturn. Future research should focus on quantifying and measuring the metabolic processes performed by individual members within the microbial community to assess their individual roles in nutrient biogeochemical cycling.

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