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Abundance and Diversity of Organohalide-Respiring Bacteria in Lake Sediments Across A Geographical Sulfur Gradient

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# Abstract

Across the U.S. Upper Midwest, a natural geographical sulfate gradient exists in lakes. Sediment grab samples and cores were taken to explore whether this sulfur gradient impacted organohalide-respiring *Chloroflexi* in lake sediments. Putative organohalide-respiring *Chloroflexi* were detected in 67 of 68 samples by quantitative polymerase chain reaction. Their quantities ranged from 3.5 × 104 to 8.4 × 1010 copies 16S rRNA genes g−1 dry sediment and increased in number from west to east, whereas lake sulfate concentrations decreased along this west-to-east transect. A terminal restriction fragment length polymorphism (TRFLP) method was used to corroborate this inverse relationship, with sediment samples from lower sulfate lakes containing both a higher number of terminal restriction fragments (TRFs) belonging to the organohalide-respiring *Dehalococcoidetes*, and a greater percentage of the TRFLP amplification made up by *Dehalococcoidetes* members. Statistical analyses showed that dissolved sulfur in the porewater, measured as sulfate after oxidation, appeared to have a negative impact on the total number of putative organohalide-respiring *Chloroflexi*, the number of *Dehalococcoidetes* TRFs, and the percentage of the TRFLP amplification made up by *Dehalococcoidetes*. These findings point to dissolved sulfur, presumably present as reduced sulfur species, as a potentially controlling factor in the natural cycling of chlorine, and perhaps as a result, the natural cycling of some carbon as well.

# Keywords

halorespiration, *Dehalococcoides*, *Dehalobacter*, *Desulfitobacterium*, sulfur cycle, chlorine cycle, carbon cycle

# Introduction

The *Chloroflexi* phylum contains several deeply branching lineages of organohalide-respiring organisms, possibly spanning several classes (Watts *et al.*, 2005; Kittelmann & Friedrich, 2008a). The *Chloroflexi* class *Dehalococcoidetes* (Löffler *et al.*, 2012) has been a subject of fairly intense study over the past 10 years because of the role that these organisms play in the dechlorination and subsequent detoxification of anthropogenic contaminants (e.g. Bedard, 2008). Indeed, the isolates of the class *Dehalococcoidetes* have all been found to be obligate anaerobic organohalide respirers (Maymó-Gatell *et al.*, 1997; Adrian *et al.*, 2000; Cutter *et al.*, 2001; Cupples *et al.*, 2003; He *et al.*, 2003, 2005; Sung *et al.*, 2006; May *et al.*, 2008; Cheng & He, 2009; Moe *et al.*, 2009; Yan *et al.*, 2009). Strains of the species *Dehalococcoides mccartyi* are the best-characterized group of *Dehalococcoidetes* and organohalide-respiring *Chloroflexi* in general, and they were the first to be isolated (Maymó-Gatell *et al.*, 1997; Löffler *et al.*, 2012). These strains, or those closely related phylogenetically, are commonly found in contaminated sites where they are presumed to have a niche dechlorinating pollutants (Hendrickson *et al.*, 2002; Müller *et al.*, 2004; Imfeld *et al.*, 2008; Scheutz *et al.*, 2008).

It has recently been hypothesized that organisms in the *Dehalococcoidetes* class and potentially other organohalide-respiring *Chloroflexi* play a larger role in ecosystems as part of the natural chlorine cycle (Adrian *et al.*, ; Bunge *et al.*, 2008; Hiraishi, 2008; Kittelmann & Friedrich, 2008a, b; Krzmarzick *et al.*, 2012). In uncontaminated soils, the quantity of 16S rRNA gene sequences related to *Dehalococcoides* species has been correlated with the fraction of organic carbon that was chlorinated (Krzmarzick *et al.*, 2012). In addition, when fed enzymatically produced organochlorines in the laboratory, *Dehalococcoides*-like organisms increased in abundance (Krzmarzick *et al.*, 2012). Nevertheless, little is known about organohalide-respiring *Chloroflexi* in the natural environment or the environmental and geochemical parameters that stimulate or inhibit their activity and/or growth.

A natural geographical sulfur gradient exists across the U.S. Upper Midwest in which lake water sulfate levels generally increase from east (Minnesota) to west (North Dakota and South Dakota) (Table 1) as a result of hydrological and geological factors (Dean & Gorham, 1976; Gorham *et al.*, 1982, 1983). Sulfite and sulfide have been observed to inhibit reductive dechlorination activity in the laboratory (Magnuson *et al.*, 1998; He *et al.*, 2005; Rysavy *et al.*, 2005; May *et al.*, 2008) and may therefore be important for controlling the activity of organohalide-respiring *Chloroflexi* in the environment. In addition, sulfide may limit the bioavailability of required trace metals, again, negatively impacting organohalide respiration. In this research, we took advantage of this geographical gradient to study the abundance of putative organohalide-respiring bacteria in uncontaminated lake sediments and determine whether porewater sulfur impacts the abundance and diversity of organohalide-respiring *Chloroflexi* in lake sediment. As anthropogenic activities such as farming and the combustion of coal alter the regional sulfur content of various water bodies, this information may become critical for understanding natural chlorine cycling and potentially the cycling of some carbon as well.

The quantity of chloride and sulfate in lake water in this study compared to historical data

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **This study** |  | **Historical** |  |
| **Lake** | **Chloride (mM)** | **Sulfate (mM)** | **Chloride (mM)** | **Sulfate (mM)** |
| Salt Lake, MN | 23.69 ± 0.00 | 79.34 ± 7.50 | 50.4 | 104.4 |
| Dry Lake, SD | 0.50 ± 0.04 | 10.01 ± 0.08 | 0.79 | 0.853 |
| Waubay Lake, SD | 0.57 ± 0.02 | 11.36 ± 0.61 | 4.681 | 48.88 |
| Lake Parmley, SD | 1.72 ± 0.02 | 2.95 ± 0.04 | 2.327 | 1.7005 |
| Richmond Lake, SD | 1.61 ± 0.15 | 3.41 ± 0.24 | 1.912 | 1.9085 |
| Sand Lake, SD | 1.64 ± 0.20 | 5.69 ± 0.08 | 1.664 | 1.869 |
| Devil's Lake, ND | 3.91 ± 0.26 | 9.05 ± 0.39 | 30.256 | 51.515 |
| Free People's Lake ND | 12.15 ± 0.50 | 20.03 ± 0.02 | 20.896 | 31.2 |
| Elbow Lake, ND | 1.10 ± 0.06 | 0.48 ± 0.14 | 0.959 | 0.905 |
| East Stump Lake, ND | 10.21 ± 0.31 | 35.57 ± 0.81 | 321.51 | 620.35 |
| Long Lake, MN | 0.27 ± 0.02 | 0.020 ± 0.000 | 0.025 | 0.0325 |
| Lake Itasca, MN | 0.02 ± 0.00 | 0.010 ± 0.000 | 0.03 | 0.0275 |
| Leech Lake, MN | 0.10 ± 0.00 | 0.023 ± 0.001 | 0.054 | 0.0445 |
| Winnibigoshish, MN | 0.13 ± 0.00 | 0.027 ± 0.002 | 0.034 | 0.055 |
| Moose Lake, MN | 0.05 ± 0.00 | 0.023 ± 0.001 | 0.023 | 0.0545 |
| Ball Club Lake, MN | 0.07 ± 0.00 | 0.025 ± 0.001 | 0.017 | 0.069 |
| Pelican Lake, MN | 0.12 ± 0.00 | 0.013 ± 0.001 | 0.031 | 0.0425 |
| Gladstone Lake, MN | 0.10 ± 0.01 | 0.004 ± 0.000 | 0.01 | 0.03 |
| Nokay Lake, MN | 0.05 ± 0.00 | 0.030 ± 0.003 | 0.021 | 0.0465 |
| Lake Mille Lacs, MN | 0.23 ± 0.00 | 0.061 ± 0.005 | 0.071 | 0.074 |

Data from Gorham *et al.,* (1982).

Mean ± standard deviation.

# Materials and methods

## Sampling

Sediment and lake water samples were gathered between November 22, and November 24, 2010. Longitude and latitude of sampling locations are indicated in Supporting Information, Table S1. The lakes chosen for sampling had significant historical data available (see Dean & Gorham, 1976; Gorham *et al.*, 1982, 1983). During sampling, ambient air temperatures ranged from −18 to −5 °C and lake water temperatures ranged from 0.2 to 6 °C. In lakes with ice cover, sampling holes were chipped with a shovel. Sediment and lake water were collected 5–25 m from the shore at lake depths of 1.5–2.5 m, and all samples were kept on ice until arrival in the laboratory. A Hydrolab DS5X (Hach) was used for temperature, pH, specific conductivity, turbidity, dissolved oxygen, and depth measurement. Lake water was sampled at a depth of 0.2 m below the surface of the lake using 1-L plastic bottles according to standardized procedures (Bordner *et al.*, 1978). Sediment cores (*n* = 4) between 12 and 30 cm deep were taken with a plastic coring apparatus and kept in the plastic core sleeve until arrival at the laboratory. Sediment grab samples (*n* = 20) were taken at a sediment depth of 0–3 cm and transferred into aluminum containers.

Upon arrival in the laboratory, sediment cores were partitioned into 2-cm sections by sliding the intact sediment core out of the bottom end of the plastic core sleeve with gentle pressure provided with a rubber stopper pressing on the top of the core and slicing off each sediment section into aluminum containers, which were then capped. Sediment samples were briefly homogenized in the aluminum containers with a pestle and large rocks were removed. During partitioning and homogenization, materials were sterilized with a 70% ethanol solution between samples. The core partitioning process resulted in 48 samples.

Sediment from the core sections and grab samples (0.25–1.0 g) was transferred into bead-beating tubes for later microbial analysis and frozen at −20 °C. Between 1 and 1.5 mL of free porewater was collected using a pipette from each of the core sections and sediment grab samples, transferred into 1.7-mL microcentrifuge tubes, and frozen at −20 °C for later chemical analysis. Both the porewater and sediment samples were exposed to the atmosphere during collection and during the homogenization of the samples. Aliquots from each of the sediment core sections and grab samples were transferred into weigh boats for percent solids and total organic matter determination.

## Chemical analysis

Anion concentrations (F−, SO−24SO4−2⁠, PO−34PO4−3⁠, Br−, Cl−, NO−3NO3−⁠) of lake water and porewater were quantified via ion chromatography (IC) on a Metrohm 761 Compact Ion Chromatograph (Metrohm US Inc). A Metrosep A Supp5 column was used for ion separation. An isocratic method was used with an eluent (3.2 mM Na2CO3, 1.0 mM NaHCO3) flow rate of 0.7 mL min−1. Milli-Q water was used as rinsing solution, and 100 mM H2SO4 was used as the regenerant. Samples were filtered through Acrodisc 32-mm syringe filters with 0.45-μm Supor membranes (Pall Corporation) prior to injection onto the column. For samples with high anion concentrations, samples were diluted with Milli-Q (Millipore) water. All 20 lake water samples and 9 of the 68 porewater samples (grab samples from Dry Lake, Waubay Lake, and the 0- to 2-cm, 4- to 6-cm, 6- to 8-cm, 8- to 10-cm, 20- to 22-cm, 22- to 24-cm, and 28- to 30-cm sections of the Sand Lake sediment core) were analyzed in duplicate by IC. Intermittent blank samples (Milli-Q water) were analyzed with IC to ensure that exogenous contamination did not occur. Standard deviations were < 15% of the mean values in all replicated analyses. Standard deviation values are shown as error bars in figures and are reported in tables.

Because porewater processing involved exposure to oxygen (pipetting, filtration, and dilution with oxygenated water), all reduced forms of dissolved sulfur (sulfide, polysulfide, and sulfite) were assumed to oxidize to sulfate. To test this assumption, additional sediment grab samples were collected from three lakes in North Dakota, including one for which detailed recent porewater sulfur speciation data were available (Zeng *et al.*, 2011, 2012). Replicate plastic centrifuge tubes were filled with sediment and porewater such that no headspace remained to maintain anaerobic conditions. Tubes were then capped and placed on ice for transportation to the laboratory. Upon arrival, tubes were transferred to an anaerobic glovebag (Coy). In one set of tubes, sulfide was analyzed as described previously (Zeng *et al.*, 2011, 2012) using the methylene blue method (Cline, 1969). The porewater samples were stored headspace-free until analysis. In the second set, a sample of lake sediment was removed from the glovebag, porewater was removed from the sample on the benchtop via pipette, as performed for the 68 lake sediment and core samples, then diluted with oxygenated Milli-Q water, and analyzed via IC for sulfate, again, as performed for the 68 initial lake sediment and core samples. The sulfate concentration of these samples was measured by IC. The error within replicate sulfate analyses ranged from 4% to 12%. The error between paired samples subjected to sulfate analysis via IC after oxidation (referred to below as ‘porewater sulfur’) and sulfide analysis was greater, 27–227%, and in some samples (those in which more sulfide was present than sulfate), it was likely that a loss of sulfur in the form of gaseous H2S occurred on the benchtop during the preparation and analysis of the oxidized samples. Sample sulfate and sulfide comparisons are presented in Table S4.

Sediment percent solids were determined by oven drying a weighed aliquot of sediment before and after drying at 105 °C. Sediment total organic matter was estimated by loss on ignition at 550 °C. All 20 sediment grab samples and selected sections of the sediment cores from Sand Lake and Gladstone Lake were sent to the Research Analytical Laboratory at the University of Minnesota for the quantification of total sediment P, K, Ca, Mg, Na, Al, Fe, Mn, Zn, Cu, B, Pb, Ni, Cr, Cd, S, and Co by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Duplicate ICP-AES analysis was performed on six of the 35 submitted samples (grab sediment from Richmond Lake, Long Lake, and Pelican Lake and the 0- to 2-cm and 24- to 26-cm sections of the Sand Lake sediment core, and the 20- to 22-cm section of the Gladstone Lake sediment core). Standard deviations of duplicates were typically < 10% of their means, but ranged up to 22%. Means and standard deviations are reported in Table S5.

## DNA extraction and quantitative polymerase chain reaction (qPCR)

DNA was extracted from wet sediment (0.25–1.0 g) using the PowerSoil DNA kit (MoBio Laboratories) according to the recommended procedure. Extracted wet sediment was determined by weight, and qPCR values were normalized to dry weight by multiplying wet weight by percent solids. For qPCR, the number of putative organohalide-respiring *Chloroflexi* 16S rRNA genes present was determined using a previously described method that focused on the *Dehalococcoides*-like spp. (Krzmarzick *et al.*, 2012), with one exception, the forward primer was replaced with a new primer designed for this study Chl1150F (5′-GGG CTA CAC ACA CGC TAC AAT GG-3′) that captured a wider fraction of the *Chloroflexi*, such as the more recently isolated *Dehalogenimonas* spp. and *Dehalobium chlorocoercia* DF-1. In addition, primer 1150F also widely targets 16S rRNA genes from the classes *Anaerolineae* and *Caldilineae*, which may or may not be organohalide-respiring organisms. The number of *Bacteria* 16S rRNA genes was determined as previously described (Krzmarzick *et al.*, 2012). Standards were prepared as described previously (Krzmarzick *et al.*, 2012) and were serially diluted with DNAse-/RNAse-free water to make nine standards containing between 100 and 1010 copies of 16S rRNA genes per reaction. For putative organohalide-respiring *Chloroflexi* 16S rRNA gene quantification, all standards were log-linear. For *Bacteria* 16S rRNA gene quantification, standards were log-linear between 105 and 1010 copies per reaction. The detection limits, inclusive of DNA extraction and percent solids, were 104 gene copies g−1 dry sediment for putative organohalide-respiring *Chloroflexi* and 107 gene copies g−1 dry sediment for *Bacteria*.

Previously published methods were used for the quantification of the organohalide-respiring *Dehalobacter* spp. and *Desulfitobacterium* spp. (Smits *et al.*, 2004). *Dehalobacter* spp. standards were prepared from an *Escherichia coli* clone containing the 16S rRNA gene of *Dehalobacter restrictus* strain DSN 29455. Eight standards ranging from 100 gene copies per reaction to 109 gene copies per reaction were prepared from serial dilutions using DNAse-/RNAse-free water. For the quantification of *Desulfitobacterium* spp., standards were prepared from an *E. coli* clone containing the 16S rRNA gene from *Desulfitobacterium hafniense* PCP-1 (ATCC 700357). Seven standards ranging from 200 to 2 × 108 gene copies per reaction were prepared from serial dilutions using DNAse-/RNAse-free water. The detection limits were 104 gene copies g−1 dry sediment for *Dehalobacter* spp. and 2 × 105 gene copies g−1 dry sediment for *Desulfitobacterium* spp.

Additionally, a previously published method was used to quantify sulfate-reducing bacteria using the *dsrA* gene (Wilms *et al.*, 2007). Standards were prepared from the dilution of genomic DNA from *Desulfovibrio vulgaris* NCIB 8303 (ATCC). Seven standards ranging from 1000 to 107 gene copies per reaction were prepared from serial dilutions using DNAse-/RNAse-free water. The detection limit was 105 gene copies g−1 dry sediment for *dsrA*.

For all qPCR analyses, melting curve analysis was performed to screen for nonspecific amplification and primer dimer formation. In addition, each sample was quantified in duplicate, and the mean was used for further statistical analyses. Standard deviations of qPCR data are shown in all figures and tables. Inefficiencies and losses resulting from DNA extraction from sediment were not determined and are not accounted for in our quantification.

## qPCR quality assurance

As mentioned above, in addition to all isolated *Dehalococcoidetes*, the primer Chl1150F broadly targets across the classes *Anaerolineae* and *Caldilineae*, which contain both isolated bacteria that are not organohalide respiring and several sequences commonly found in organohalide-respiring mixed cultures. The reverse primer used, Dhc1286R (from Krzmarzick *et al.*, 2012), is conversely specific for *Dehalococcoides* and contains mismatches for all other isolated and non-organohalide-respiring *Chloroflexi*. Thus, the qPCR method used would not likely detect all *Dehalococcoidetes* and may detect fractions of the classes *Anaerolineae* and *Caldilineae* that contain unknown physiologies and whose organohalide-respiring activity is speculative (Watts *et al.*, 2005; Imfeld *et al.*, 2011). To test the specificity of the qPCR amplification for putative organohalide-respiring *Chloroflexi*, clone libraries were constructed from the amplification products of three samples: the 2- to 4-cm-depth section of the sediment core from Sand Lake, SD, the 2- to 4-cm-depth section of the sediment core from Long Lake, MN, and the grab sediment section from East Stump Lake, ND. Of 48 clones sequenced, 45 unique sequences were obtained. These sequences aligned with known organohalide-respiring *Chloroflexi* and with clone sequences obtained from NCBI's Blast Database that came from dehalogenating mixed cultures (see Fig. S2). Clone library analysis (see Fig. S2) verified that the qPCR method amplified sequences that likely group phylogenetically with the class *Dehalococcoidetes*, whose isolated members are all obligate organohalide respirers, as well as the closely related classes of *Anaerolineae* and *Caldilineae*, which are merely speculatively associated with organohalide respiration. Because of this, the term ‘putative organohalide-respiring *Chloroflexi*’ is used in this manuscript to refer to the organisms quantified by qPCR.

To ensure that inhibition did not occur with the qPCR method used, serial dilutions (10- and 100-fold) of the DNA extracts for ten samples (grab samples from Salt Lake, Waubay Lake, Lake Parmley, Richmond Lake, Sand Lake, Dry Lake, East Stump Lake, Devil's Lake, Elbow Lake, and Free People's Lake) were prepared and tested via qPCR for *Bacteria* and putative organohalide-respiring *Chloroflexi*. The calculated quantities of both *Chloroflexi* and *Bacteria* 16S rRNA genes g−1 dry sediment did not change significantly (< ±10%) as a result of dilution; thus, PCR inhibition from compounds in the DNA extract was not a factor in our work.

## Terminal restriction fragment length polymorphism analysis

For terminal restriction fragment length polymorphism (TRFLP), *Chloroflexi* 16S rRNA genes were amplified with two primers designed for this study: Dhc553F (5′-CCG GCT TAA CCG GGA CG WGT-3′) and Chl1150R (5′-CCA TTG TAG CGT GTG TGT AGC CC-3′). The primer Dhc553 is specific for *Dehalococcoides* spp., while Chl1150R more generally targets the classes *Dehalococcoidetes, Anaerolineae*, and *Caldilineae*. The method is therefore expected to be biased toward *Dehalococcoides*-like sequences, and in samples with high numbers of *Dehalococcoides*-like sequences, the fraction of the TRFLP amplification belonging to *Dehalococcoidetes* is expected to be large. In samples with scarce *Dehalococcoides*-like sequences, the proportion of the amplification belonging to non-*Dehalococcoidetes* classes is expected to be larger. PCR mixture (30 μL) contained 1× reaction buffer (Promega), 1 mM MgCl2, 2.5 μg BSA, 0.2 mM each dNTP, 0.5 mM each primer, 1.5 U *Taq* DNA polymerase, and 1 μL of DNA extract. The thermocycling procedure contained an initial denaturing step at 95 °C for 5 min, 30 cycles of 95 °C for 45 s, 58 °C for 30 s, 72 °C for 1 min, and final extension step of 72 °C for 5 min. PCR amplification products were screened on 1% agarose gels for proper size (~ 600 bp) to ensure the specificity of amplification, and PCR products were cleaned using the GeneClean II kit (MP Biomedical). Cleaned product was used as template for a second PCR amplification, performed as before except with a carboxyfluorescein-labeled Chl1150R primer. PCR products were cleaned again and digested with 2 U each of TaqαI, RsaI, BamHI, and 1× Buffer 4 (New England Biolabs) at 37 °C for 2 h, then 65 °C for 1 h, followed by 20 min of incubation at 80 °C for deactivation. Fragment analysis was performed by the Biomedical Genomics Center (University of Minnesota) on an ABI 3730xl capillary instrument with GeneMapper Software. MapMarker 1000 was used as a size standard. The results of the TRFLP were analyzed using PEAKSCANNER software v1.0 (Applied Biosystems). Peaks above 50 fluorescence units and between 50 and 600 bp in length were used in the analysis. Peaks were manually binned with Microsoft Excel. DNA extracts from each of the 20 grab sediment samples were amplified, digested, and analyzed by capillary electrophoresis in duplicate. Peaks present in only one of the duplicate samples were removed, the relative peak areas were averaged, and the peaks with peak areas < 0.5% were removed.

To match the terminal restriction fragments (TRFs) from the TRFLP analysis to the *Dehalococcoidetes* class or other *Chloroflexi* classes, clone libraries were constructed from the amplification products of Dry Lake, Sand Lake, and Leech Lake (see Table S2 and S3, Fig. S2). From these three clone libraries, a total of 11 TRFs were identified out of 29. Smaller clone libraries from an additional four samples (grab samples from Salt Lake, Long Lake, Devils Lake, and Ball Club Lake) produced a classification of four additional TRFs, for a total of 15 TRFs of 29. These 15 TRFs represented between 82% and 99% of the total peak area for each sample with an average of 92% for all samples. A total of eight TRFs grouped within the *Dehalococcoidetes* class, while seven grouped outside of this class. The unique sequences were deposited into NCBI's GenBank (accession numbers JQ691831–JQ691944).

## Statistical analysis

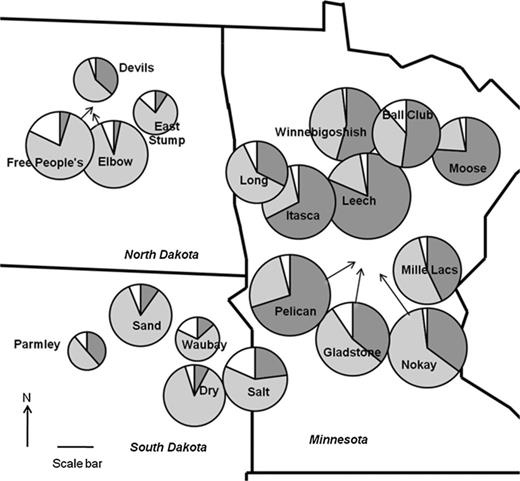
Statistical analyses [Spearman's rank coefficient test and principal component analysis (PCA)] were performed using STATA/IC 10.1 software. The nonparametric Spearman's rank coefficient test was used to compare the relationships between parameters. Two-tailed Student's *t*-tests were performed with Microsoft Excel to compare TRFLP parameters between sediments containing high porewater sulfur (> 1.0 mM) and low porewater sulfur (< 1.0 mM). A *P* value < 0.05 was considered significant.

# Results

## Distribution of organohalide-respiring Chloroflexi along a geographical sulfate gradient

Putative organohalide-respiring *Chloroflexi* were found in nearly all of the 20 grab samples and 48 core sections analyzed (67 of 68 total samples). In addition, their quantities in the lake sediment samples were quite high, ranging from 3.5 × 104 to 8.5 × 1010 copies 16S rRNA genes g−1 dry sediment and averaging 1.6% of the total *Bacteria* 16S rRNA genes in the sediment (Table S5). The abundance of putative organohalide-respiring *Chloroflexi* 16S rRNA genes correlated with the abundance of *Bacteria* 16S rRNA genes in the sediment samples (Spearman's ρ = 0.92, *P* < 0.001).

Lake water sulfate concentrations are presented, along with historical data (Gorham *et al.*, 1983), in Table 1. Interestingly, there was a strong geographical pattern with respect to the number of putative organohalide-respiring *Chloroflexi* in lake sediments, increasing from west to east in our sampling area, inverse to the geographical lake water sulfate gradient, which increased from east to west (Fig. 1, Table 1). These results corroborate our previous findings (Krzmarzick *et al.*, 2012) that the putative organohalide-respiring *Chloroflexi* are members of uncontaminated ecosystems and may therefore be an important part of chlorine and carbon cycles. TRFLP, augmented by clone libraries, was also performed on 20 sediment grab samples to further corroborate the qPCR data. Clone libraries indicated that sequences of the *Dehalococcoidetes* class and members of the related *Anaerolineae* and *Caldilineae* classes were amplified (Fig S1). These clone libraries were used to match the TRFs to subphyla of *Chloroflexi* or to members of the *Dehalococcoidetes* class, the only class containing isolated organohalide-respiring *Chloroflexi* (see Supporting Information). It was hypothesized that if organohalide respirers were common in a given sample, the relative fraction of TRFs belonging to the *Dehalococcoidetes* class would be large in comparison with the relative fraction of TRFs from outside *Dehalococcoidetes*. In contrast, if organohalide respirers were relatively rare in a sample, the fraction of amplicons belonging to *Dehalococcoidetes* should be smaller and the fraction of amplicons from other subphyla should be greater.

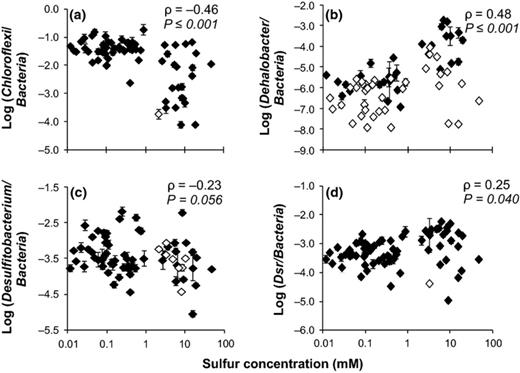


The quantity of putative organohalide-respiring *Chloroflexi* determined by qPCR is shown by the diameter of the circle, with the scale bar equaling 5 logarithmic units of *Chloroflexi* 16S rRNA gene copies g−1 dry sediment. Standard deviations are within the width of the line making the circle and are available in Table S5. The percentage of the TRFLP amplification determined to be *Dehalococcoidetes* (dark gray), other *Chloroflexi* (light gray), and undetermined (white) by TRFLP analysis is shown by the pie charts. Positions of circles, or arrows (when present), are approximate locations of the lake from which samples were taken. For Richmond Lake (not shown), no *Chloroflexi* 16S rRNA genes were detected in TRFLP analysis and the quantification of putative organohalide-respiring *Chloroflexi* was 4.55 ± 0.19 logarithmic units of 16S rRNA genes g−1 dry sediment (just above detection limit). Lake water sulfate concentrations generally increased from east to west.

Indeed, the TRFLP analysis corroborated the qPCR findings (Fig. 1). The percentage of the amplicons belonging to members of the *Dehalococcoidetes* class was strongly dependent on geography, with fractions of *Dehalococcoidetes* members increasing as one moved from west to east along a transect of generally decreasing lake water sulfate concentration (Fig. 1, Table 1). Additionally, the average number of TRFs belonging to *Dehalococcoidetes* followed a similar trend, with higher numbers of TRFs found in the sediment from the lakes to the east containing lower water concentrations of sulfate (Table S3).

## Effect of porewater chemistry on the relative abundance and diversity of putative organohalide-respiring *Chloroflexi* and on the abundance of *Desulfitobacterium* and *Dehalobacter*

The distinct geographical distribution of organohalide-respiring *Chloroflexi* was further investigated by determining whether the numbers and diversity of putative organohalide-respiring *Chloroflexi* correlated with sediment porewater sulfur concentration. Because of the correlation between the abundance of *Chloroflexi* and *Bacteria* 16S rRNA genes, the number of putative organohalide-respiring *Chloroflexi* 16S rRNA genes present in a given sample was normalized to total *Bacteria* 16S rRNA genes (*Chloroflexi*/*Bacteria*). *Chloroflexi*/*Bacteria* was found to be significantly and inversely correlated with the porewater sulfur concentration (Fig. 2) (Spearman's ρ = −0.46, *P* < 0.001). Upon inspection of the data, however (Fig. 2), this correlation appeared to be the result of a ‘switch’ that occurred at a porewater sulfur concentration of 1 mM. No signification correlation existed between subsets of the data in which only > 1 mM or < 1 mM sulfur porewater concentrations were included.



The quantity of (a) *Chloroflexi* 16S rRNA gene copies, (b) *Dehalobacter* 16S rRNA gene copies, (c) *Desulfitobacterium* 16S rRNA gene copies, and (d) *dsrA* gene copies (all normalized to *Bacteria* 16S rRNA gene copies) in dry sediment vs. the sulfur porewater concentrations for all 68 sediment grab samples and core sections. The black symbols indicate measurements above the minimum detection limits, while the white diamonds represent samples for which the gene copy numbers for *Chloroflexi, Desulfitobacterium, Dehalobacter*, or *dsrA* were below the detection limit. For those values below the detection limit, the value shown in the plot represents the logarithm of the associated detection limit normalized by the quantity of *Bacteria* 16S rRNA gene copies measured in that sample. The Spearman's ρ and associated *P* values for each association are given and are inclusive of the samples below the detection limit (at a value equal to the associated detection limit normalized by *Bacteria* 16S rRNA gene copies). Error bars represent standard deviations.

In addition, the percentage of TRFLP amplicons belonging to members of the *Dehalococcoidetes* class and the average number of TRFs belonging to *Dehalococcoidetes* were both strongly and statistically affected by porewater sulfur concentrations. Sediments with porewater sulfur concentrations < 1 mM contained higher fractions of *Dehalococcoidetes* members (an average of 55% compared to 16% in the > 1 mM porewater sulfur samples) (Student's *t*-test *P* < 0.001) and a higher number of TRFs belonging to *Dehalococcoidetes* (an average of 5.2 TRFs compared to 3.4 TRFs in the > 1 mM porewater sulfur samples) (Student's *t*-test *P* = 0.005) (Fig. 1, Table S3).

Porewater chloride was also negatively associated with *Chloroflexi*/*Bacteria*. Nevertheless, because chloride and sulfur strongly correlated with each other (Spearman's ρ = 0.92, *P* < 0.001), we tested that sulfur rather than chloride was the parameter of interest. When porewater sulfur (oxidized and measured as sulfate with IC, see Methods) was normalized by the total porewater anion concentration (the sum of sulfate, bromide, chloride, phosphate, and nitrate), the statistical relationships with *Chloroflexi*/*Bacteria* retained its significance and direction (Spearman's ρ = −0.28, *P* = 0.020). When porewater chloride was similarly normalized, however, the relationship between *Chloroflexi*/*Bacteria* and normalized porewater chloride was positive and statistical significance was lost (Spearman's ρ = 0.14, *P* = 0.230). This suggests that porewater sulfur, not chloride, was indeed the parameter of interest in these correlations.

*Desulfitobacterium* and *Dehalobacter* populations, both known to respire organohalides, and the *dsrA* gene, involved in sulfate reduction, were also enumerated in lake sediment samples*. Dehalobacter* spp. was measured because it is the only group outside of the organohalide-respiring *Chloroflexi* that are obligate organohalide respirers (Holliger *et al.*, 1998; Sun *et al.*, 2002) and may therefore have a similar niche to organohalide-respiring *Chloroflexi*. *Dehalobacter* 16S rRNA genes normalized to *Bacteria* 16S rRNA genes (*Dehalobacter*/*Bacteria*) positively correlated with porewater sulfur concentration in these samples (Spearman's ρ = 0.48, *P* < 0.001) (Fig. 2 and Table S5). The abundance of *Dehalobacter* 16S rRNA genes detected (1.5 × 104 to 1.5 × 106 gene copies g−1 dry sediment), however, was generally low in comparison with the *Chloroflexi*, with a large number of samples below the detection limit (37 of 68 samples); therefore, additional work is needed to test and understand any relationship between *Dehalobacter* spp. and sulfur.

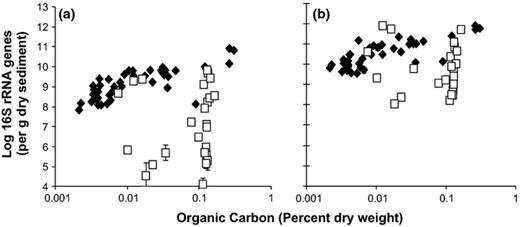
*Desulfitobacterium* spp. were measured because in addition to being capable of organohalide respiration (Nonaka *et al.*, 2006), *Desulfitobacterium* spp. are also able to reduce sulfate and may therefore have an ecological advantage in higher sulfur sediments. When normalized to *Bacteria* 16S rRNA genes, *Desulfitobacterium* 16S rRNA genes negatively correlated with porewater sulfur (Spearman's ρ = −0.23 *P* = 0.056), although not significantly, and did not correlate with *Chloroflexi*/*Ba*c*teria* (Spearman's ρ = 0.16, *P* = 0.184). This suggested that despite a theoretical advantage stemming from the ability to reduce sulfate, *Desulfitobacterium* spp. did not appear to have a competitive advantage over organohalide-respiring *Chloroflexi* in sediments containing higher porewater sulfur.

Finally, to test whether the correlation between putative organohalide-respiring *Chloroflexi* and porewater sulfur was simply a result of sulfate-reducing bacteria outcompeting organohalide respirers for nutrients or carbon, the sulfate-reducing gene *dsrA* was also measured using qPCR. The number of *dsrA* gene copies normalized to *Bacteria* did positively correlate with porewater sulfur (ρ = 0.25, *P* = 0.040) (Fig. 2 and Table S5) but did not correlate with the abundance of *Chloroflexi*/*Bacteria* (Spearman's ρ = −0.02, *P* = 0.852). These results suggest that, as expected, sulfate-reducing bacteria may have a competitive advantage in high sulfate lakes, yet this advantage does not, in and of itself, appear to impact the relative abundance of organohalide-respiring *Chloroflexi* in the sediment.

Impact of other geochemical parameters on organohalide-respiring *Chloroflexi*

PCA was performed for the sediments in which sediment and porewater chemistry was measured by ICP-AES to further investigate possible covariance with putative organohalide-respiring *Chloroflexi* (Fig S3). The elements measured in the sediment (Table S5) generally covaried with each other, but not with porewater sulfur or *Chloroflexi*/*Bacteria*. Nevertheless, because the ICP-AES method measured both the bioavailable and nonbioavailable forms of the elements, such potential relationships require more investigation, particularly because the total concentration of a given element may have been quite different than the concentration of that element in a bioavailable form or in a given critical redox state.

The effect of TOC was investigated as well. Organic carbon may be particularly important for organohalide-respiring bacteria, either directly as a carbon source or as a precursor to the likely electron donors hydrogen and/or acetate (formed by fermentative and acetogenic bacteria) (e.g. Heimann *et al.*, 2006). Although the quantity of both *Bacteria* and putative organohalide-respiring *Chloroflexi* 16S rRNA genes correlated strongly with the organic matter content of the sediment samples in low porewater sulfur lakes (< 1 mM) (Spearman's ρ = 0.77, *P <* 0.001; Spearman's ρ = 0.74, *P <* 0.001, Fig. 3), these correlations did not exist in the samples containing high (> 1 mM) porewater sulfur concentrations (Spearman's ρ = 0.34, *P =* 0.10 for *Chloroflexi* and Spearman's ρ = 0.31, *P =* 0.15 for *Bacteria*) or when all of the low- and high-sulfur samples were pooled (Spearman's ρ = 0.08, *P =* 0.50 for *Chloroflexi* and Spearman's ρ = 0.13, *P =* 0.13 for *Bacteria*). Additionally, *Chloroflexi*/*Bacteria* did not correlate with TOC in low porewater sulfur samples (Spearman's ρ = 0.19, *P =* 0.22), high porewater sulfur samples (Spearman's ρ = 0.37, *P =* 0.08), or when all porewater samples were tested (Spearman's ρ = −0.18, *P =* 0.14). Additionally, no correlation existed between TOC and *Dehalobacter*/*Bacteria* (Spearman's ρ = 0.16, *P* = 0.18) or between *Desulfitobacterium*/*Bacteria* (Spearman's ρ = −0.20, *P =* 0.10). Thus, TOC did not appear to selectively increase the abundance of organohalide respirers relative to the general *Bacteria* population in our samples.



The quantity of (a) *Chloroflexi* 16S rRNA genes copies and (b) *Bacteria* 16S rRNA genes copies in the dry sediment vs. organic carbon in low sulfur lake sediments (< 1.0 mM porewater sulfur, black diamonds) and higher sulfur lake sediments (> 1.0 mM porewater sulfur, white squares).

# Discussion

Although organohalide-respiring *Chloroflexi* likely play an important role in the cycling of chlorine and are beneficial for bioremediation applications, little is known about their natural physiology. Our results indicate that porewater sulfur concentration may have an important negative effect on the abundance of these organisms at concentrations > 1 mM. In our analysis, porewater sulfur concentration was measured via the oxidation of porewater followed by IC analysis of sulfate; therefore, it was an aggregation of the concentration of sulfate and the reduced forms of sulfur in the porewater. Because of the potential loss of gaseous H2S during oxidation, this value may have been an underestimate of total sulfur by as much as two times (Table S4). Based on literature values of sulfate and sulfide concentrations in pooled sediment porewater from two North Dakota prairie pothole lake sediment cores (1.02 ± 0.01 and 2.37 ± 0.03 mM for sulfate and sulfide, respectively, in Lake PI and 0.37 ± 0.03 and 2.06 ± 0.05 mM for sulfate and sulfide, respectively, in Lake P8) (Zeng *et al.*, 2011, 2012), the presence of sulfate in our porewater samples is likely, but it is also likely that sulfide was the dominant sulfur species in the samples. Indeed, the porewater sulfur concentration that appeared to negatively impact the abundance of putative organohalide-respiring *Chloroflexi* in the research presented herein is similar to observations by others regarding the concentration of sulfide (5–10 mM) and sulfite (0.5–2 mM) that inhibits organohalide respiration (Magnuson *et al.*, 1998; He *et al.*, 2005; May *et al.*, 2008). The lack of sulfur speciation data in our porewater samples, which precludes our ability to distinguish between sulfate, sulfide, sulfite, and polysulfides in the porewater, and the potential loss of gaseous H2S in some samples, is likely to have resulted in the high degree of scattering in the data at porewater sulfur concentrations > 1 mM.

Although the quantity of atmospheric sulfur precipitation in the United States and Europe has been declining, it has been increasing in developing countries such as China (Kahl *et al.*, 2004; EIA, 2008; Mitchell & Likens, 2011). Furthermore, the use of sulfur compounds in fertilizers has been on the rise (e.g. Chien *et al.*, 2011 and references therein) and perhaps is the cause of the unique increase in sulfate measured in this study in the lakes from South Dakota. These anthropogenic pressures may therefore increase sulfur concentrations in particular environments, not only impacting natural attenuation in contaminated sites, but also impacting chlorine cycling in uncontaminated environments as well. Interestingly, natural organochlorines also tend to be recalcitrant (Winterton, 2000; Redon *et al.*, 2011), suggesting that sulfur may also impact carbon cycling. Further research is needed on the effect of geochemical parameters on reductive dehalogenase genes in the environment, on the wider role of other organohalide-respiring organisms, such as those in the *Firmicutes* phylum, on the chlorine cycle, and on the role of organohalide-respiring organisms, in general on carbon cycling.

Finally, the correlation between putative organohalide-respiring *Chloroflexi* and *Bacteria* is not surprising and suggests that, as observed by others (e.g. Seshadri *et al.*, 2005; He *et al.*, 2007), putative organohalide-respiring *Chloroflexi* may have a nutritional dependence on other organisms. Alternatively, there may be habitat characteristics that are generally favorable or unfavorable for microbial growth, resulting in niches in which both the organohalide-respiring *Chloroflexi* and *Bacteria* generally thrive. This apparent synergism also requires more research, particularly as it impacts the cycling of chlorine and carbon in the environment.

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# Supporting Information

[Fig. S1. Phylogenetic tree of sequences from clone libraries show the relationship of the sequences analyzed in the TRFLP method within the Chloroflexi.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605052020&Signature=HLdYBoHy7UlE7LfDydxFzOZ0NPe~j7WzDaQtaWbRfsWCQSGu8D7k8-K3rs7~mbLW1ia6VImI5zSjsh4CVBwhrDC-XrqyBZYFLZARVEEEHN~KeKW4AYz4ejDTax9QIrwuydtrV6fDd6THsZALA1trPMEOIZehnv7QnTkIvZrN-1CKr3MDmzzuoUkP85PYbqISmBKelzvUD1XMi8iwnRKCBjT~hZdPG71keSOX4AIrTltJ-xVBKsDZqRm44PIvuomuVjAhTUX~LKzrXMbfjahDERj2R5v2Sr4~YXPseRHSS0Htzske9MSkDUroZ76DM~HMrX5fjk-xjb1YCIBfEbyLMw__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

[Fig. S2. Relationship of clones obtained from qPCR amplification using the primers Chlflx1150F and Dhc1286R in this study.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605052020&Signature=HLdYBoHy7UlE7LfDydxFzOZ0NPe~j7WzDaQtaWbRfsWCQSGu8D7k8-K3rs7~mbLW1ia6VImI5zSjsh4CVBwhrDC-XrqyBZYFLZARVEEEHN~KeKW4AYz4ejDTax9QIrwuydtrV6fDd6THsZALA1trPMEOIZehnv7QnTkIvZrN-1CKr3MDmzzuoUkP85PYbqISmBKelzvUD1XMi8iwnRKCBjT~hZdPG71keSOX4AIrTltJ-xVBKsDZqRm44PIvuomuVjAhTUX~LKzrXMbfjahDERj2R5v2Sr4~YXPseRHSS0Htzske9MSkDUroZ76DM~HMrX5fjk-xjb1YCIBfEbyLMw__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

[Fig. S3. Principal component analysis of qPCR data, sediment characterization data, and porewater characterization data for all samples subject to ICP-AES (35 grab samples and core sections).](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605052020&Signature=HLdYBoHy7UlE7LfDydxFzOZ0NPe~j7WzDaQtaWbRfsWCQSGu8D7k8-K3rs7~mbLW1ia6VImI5zSjsh4CVBwhrDC-XrqyBZYFLZARVEEEHN~KeKW4AYz4ejDTax9QIrwuydtrV6fDd6THsZALA1trPMEOIZehnv7QnTkIvZrN-1CKr3MDmzzuoUkP85PYbqISmBKelzvUD1XMi8iwnRKCBjT~hZdPG71keSOX4AIrTltJ-xVBKsDZqRm44PIvuomuVjAhTUX~LKzrXMbfjahDERj2R5v2Sr4~YXPseRHSS0Htzske9MSkDUroZ76DM~HMrX5fjk-xjb1YCIBfEbyLMw__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

[Table S1. Longitude and latitude of sampling location for the lakes sampled in this study.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605052020&Signature=HLdYBoHy7UlE7LfDydxFzOZ0NPe~j7WzDaQtaWbRfsWCQSGu8D7k8-K3rs7~mbLW1ia6VImI5zSjsh4CVBwhrDC-XrqyBZYFLZARVEEEHN~KeKW4AYz4ejDTax9QIrwuydtrV6fDd6THsZALA1trPMEOIZehnv7QnTkIvZrN-1CKr3MDmzzuoUkP85PYbqISmBKelzvUD1XMi8iwnRKCBjT~hZdPG71keSOX4AIrTltJ-xVBKsDZqRm44PIvuomuVjAhTUX~LKzrXMbfjahDERj2R5v2Sr4~YXPseRHSS0Htzske9MSkDUroZ76DM~HMrX5fjk-xjb1YCIBfEbyLMw__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

[Table S2. Summary of results from clone library analysis TRFLP primers between Dehalococcoidetes (Ddts) class and other Chloroflexi classes (Other).](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605052020&Signature=HLdYBoHy7UlE7LfDydxFzOZ0NPe~j7WzDaQtaWbRfsWCQSGu8D7k8-K3rs7~mbLW1ia6VImI5zSjsh4CVBwhrDC-XrqyBZYFLZARVEEEHN~KeKW4AYz4ejDTax9QIrwuydtrV6fDd6THsZALA1trPMEOIZehnv7QnTkIvZrN-1CKr3MDmzzuoUkP85PYbqISmBKelzvUD1XMi8iwnRKCBjT~hZdPG71keSOX4AIrTltJ-xVBKsDZqRm44PIvuomuVjAhTUX~LKzrXMbfjahDERj2R5v2Sr4~YXPseRHSS0Htzske9MSkDUroZ76DM~HMrX5fjk-xjb1YCIBfEbyLMw__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

[Table S3. Summary table of TRFLP results for each grab sediment sample.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605052020&Signature=HLdYBoHy7UlE7LfDydxFzOZ0NPe~j7WzDaQtaWbRfsWCQSGu8D7k8-K3rs7~mbLW1ia6VImI5zSjsh4CVBwhrDC-XrqyBZYFLZARVEEEHN~KeKW4AYz4ejDTax9QIrwuydtrV6fDd6THsZALA1trPMEOIZehnv7QnTkIvZrN-1CKr3MDmzzuoUkP85PYbqISmBKelzvUD1XMi8iwnRKCBjT~hZdPG71keSOX4AIrTltJ-xVBKsDZqRm44PIvuomuVjAhTUX~LKzrXMbfjahDERj2R5v2Sr4~YXPseRHSS0Htzske9MSkDUroZ76DM~HMrX5fjk-xjb1YCIBfEbyLMw__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

[Table S4. Summary of data comparing the methodology for porewater ‘sulfur’ used in this study to measured porewater sulfide.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605052020&Signature=HLdYBoHy7UlE7LfDydxFzOZ0NPe~j7WzDaQtaWbRfsWCQSGu8D7k8-K3rs7~mbLW1ia6VImI5zSjsh4CVBwhrDC-XrqyBZYFLZARVEEEHN~KeKW4AYz4ejDTax9QIrwuydtrV6fDd6THsZALA1trPMEOIZehnv7QnTkIvZrN-1CKr3MDmzzuoUkP85PYbqISmBKelzvUD1XMi8iwnRKCBjT~hZdPG71keSOX4AIrTltJ-xVBKsDZqRm44PIvuomuVjAhTUX~LKzrXMbfjahDERj2R5v2Sr4~YXPseRHSS0Htzske9MSkDUroZ76DM~HMrX5fjk-xjb1YCIBfEbyLMw__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

[Table S5. Data collected in this study not presented elsewhere.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605052020&Signature=HLdYBoHy7UlE7LfDydxFzOZ0NPe~j7WzDaQtaWbRfsWCQSGu8D7k8-K3rs7~mbLW1ia6VImI5zSjsh4CVBwhrDC-XrqyBZYFLZARVEEEHN~KeKW4AYz4ejDTax9QIrwuydtrV6fDd6THsZALA1trPMEOIZehnv7QnTkIvZrN-1CKr3MDmzzuoUkP85PYbqISmBKelzvUD1XMi8iwnRKCBjT~hZdPG71keSOX4AIrTltJ-xVBKsDZqRm44PIvuomuVjAhTUX~LKzrXMbfjahDERj2R5v2Sr4~YXPseRHSS0Htzske9MSkDUroZ76DM~HMrX5fjk-xjb1YCIBfEbyLMw__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

# Supplementary data

**[Fig. S1.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)**[Phylogenetic tree of sequences from clone libraries show the relationship of the sequences analyzed in the TRFLP method within the](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)*[Chloroflexi](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)*[.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

**[Fig. S2.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)**[Relationship of clones obtained from qPCR amplification using the primers Chlflx1150F and Dhc1286R in this study.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

**[Fig. S3.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)**[Principal component analysis of qPCR data, sediment characterization data, and porewater characterization data for all samples subject to ICP-AES (35 grab samples and core sections).](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

**[Table S1.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)**[Longitude and latitude of sampling location for the lakes sampled in this study.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

**[Table S2.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)**[Summary of results from clone library analysis TRFLP primers between](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)*[Dehalococcoidetes](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)*[(](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)*[Ddts](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)*[) class and other](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)*[Chloroflexi](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)*[classes (Other).](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

**[Table S3.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)**[Summary table of TRFLP results for each grab sediment sample.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

**[Table S4.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)**[Summary of data comparing the methodology for porewater ‘sulfur’ used in this study to measured porewater sulfide.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)

**[Table S5.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)**[Data collected in this study not presented elsewhere.](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/femsec/84/2/10.1111_1574-6941.12059/2/fem12059-sup-0001-FigureS1-S3-TableS1-S5.docx?Expires=1605205779&Signature=ntvWmQMNg3qGTvJTkuTTmUV6PbnImef0kYZcEo~GVTHaFuTz8ShVPFZYFU~sFdMdNYm5YqBDTjM8pVbfK2rrcBKjC51ZL7dt95n-OCdZfXI0Iwwzwwgp6dF~tM3Jqghnwyec88uqrnbropjQ91toTuEZan2W-DHHVcOLNYYcqz9XU97Jl9lE4WByO41gN5la5Wc2XbFylZxnwd2aw4G6-iDHikzOX3QKVMtiMsGbzzRdlSOtPepidYP~CDYrIS8N3jMMVK~1kOZZE6vhSrg2uLN77zw7bwIMk6etBTXb4wIqFYQJH1LE1irj7vD69FBzZ0darKRPwJqExqs-w6~v1g__&Key-Pair-Id=APKAIE5G5CRDK6RD3PGA)