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Ubiquitous and significant anaerobic oxidation of methane in freshwater lake sediments

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ABSTRACT

Anaerobic oxidation of methane (AOM) is a microbial process that consumes dissolved methane (CH_4) in anoxic sediments and soils and mitigates CH_4 release to the atmosphere. The degree to which AOM limits global biospheric CH_4 emissions is not fully understood. In marine sediments, where the process was first described, AOM is responsible for oxidizing >90% of the CH_4 produced. More recently, AOM has been observed in soils, peatlands, and freshwater ecosystems. In lakes, where sediment anoxia, organic carbon turnover, and CH_4 production are common, AOM is not well studied but could represent a significant CH_4 sink and constraint on emissions. Here, we present evidence for the occurrence of AOM in the sediment of thirteen lakes that span a global climatic and trophic gradient. We further quantified and modeled AOM patterns and studied potential microbial controls of AOM using laboratory incubations of sediment and stable isotope measurements in three of the thirteen lakes. We demonstrate that AOM is widespread in freshwater lake sediments and accounts for 29%–34% (95% confidence interval) of the mean total CH_4 produced in surface and near-surface lake sediments.

1. Introduction

Anaerobic oxidation of methane (AOM) has been demonstrated in a variety of freshwater ecosystems (Deutzmann and Schink, 2011; Eller et al., 2005; Schubert et al., 2010; Segarra et al. 2013, 2015; Sivan et al., 2011; Smemo and Yavitt, 2007), including lakes and reservoirs, which contribute approximately 13% of global atmospheric methane (CH_4) emissions (Saunois et al., 2016), despite representing only 3.7% of global land cover (Verpoorter et al., 2014). Previous laboratory incubation studies have identified AOM rates in lake sediments ranging from 0.01 to 100 nmol cm^{-3} d^{-1} (Deutzmann and Schink, 2011; Deutzmann et al., 2014; Nordi et al., 2013; Sivan et al., 2011; Zehnder and Brock, 1980) (Fig. 1a), or up to two orders of magnitude less than the 1–800 nmol cm^{-3} d^{-1} CH_4 production ranges estimated for those and other lake sediments (Adler et al., 2011; Deutzmann and Schink, 2011; Nordi and Thamdrup, 2014; Nordi et al., 2013; Schulz et al., 1997; Zehnder and Brock, 1980) (Fig. 1b). AOM in lakes might therefore represent an important constraint on global greenhouse gas emissions. However, the relative importance of the process among varying lake ecosystems and the specific mechanisms involved have not been determined.

Studies using amended incubations (Beal et al., 2009; Deutzmann and Schink, 2011; Deutzmann et al., 2014; Segarra et al., 2013; Zehnder and Brock, 1980) or in situ determinations of potential electron acceptor profiles (Nordi et al., 2013; Segarra et al., 2015; Smemo and Yavitt, 2007; Zehnder and Brock, 1980) have provided evidence for the same suite of electron acceptors and redox reactions that are often associated with AOM in both freshwater and marine ecosystems, including sulfate (SO_4^{2-}) reduction (Zehnder and Brock, 1980), nitrite (NO_2^-) or nitrate (NO_3^-) reduction (Deutzmann et al., 2014), Fe (III) and Mn (IV) reduction (Nordi et al., 2013; Sivan et al., 2011), or humic substances reduction (Scheller et al., 2016; Smemo and Yavitt 2007, 2011; Valenzuela et al.,

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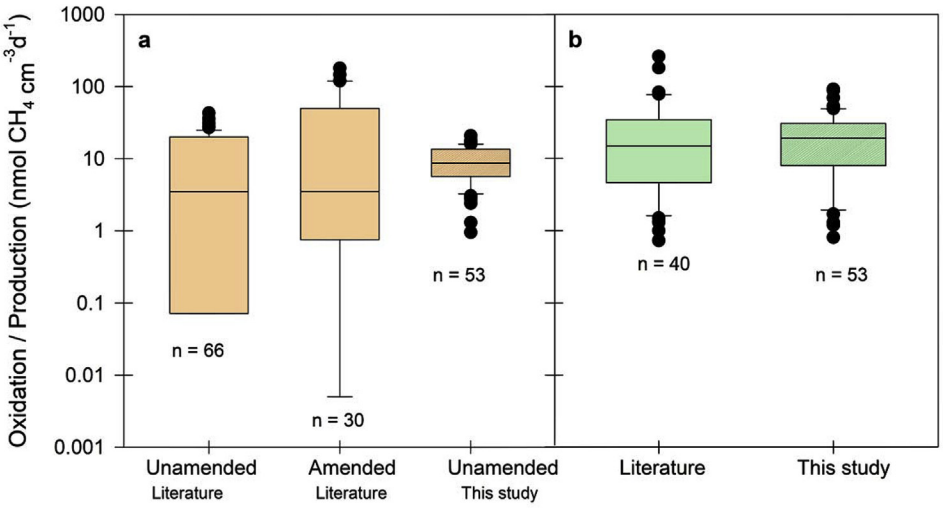

Fig. 1. Lake sediment AOM (a) and CH_4 production (b) rates reported in the literature, amended or unamended with electron acceptors, and those observed in the present study (crosshatched boxes). Data are from Doughnut, Vault, and Dagow Lake sediment cores. Boxes represent the interquartile range showing 10th and 90th percentile data, whereas whiskers are the error around the median. Open circles show outlier data. n = number of data points. No significant differences were observed among AOM or CH_4 production rates. References used in the meta-analysis are listed in Table S2.

2017). Nevertheless, many of these electron acceptors, such as SO_4^{2-} and NO_2^-/NO_3^-, are lacking in many freshwater lake sediments or have background concentrations too low for the process to be thermodynamically favorable (Alperin and Reeburgh, 1984; Smemo and Yavitt, 2007). We conducted a meta-analysis of results from previous reports and found no significant difference in AOM rates (AOMR) between the presence and absence of a range of exogenous electron acceptors (Fig. 1a). The exact role and nature of AOM electron acceptors remain uncertain at best.

AOM organisms - methanogen-like anaerobic methanotrophic (ANME) archaea, belonging to clades of ANME-1, ANME-2a,b,c, and ANME-3 - are well known to perform AOM in syntrophic association with sulfate-reducing bacteria (Knittel and Boetius, 2009; Wegener et al., 2016) or might be linked to Fe(III) and Mn(IV) reduction (Beal et al., 2009; Chang et al., 2012; He et al., 2018). Likewise, *Candidatus* Methylomirabilis oxyfera, a member of the NC10 phylum, and *Candidatus* Methanoperedens nitroreducens, an ANME-2d subclade, are capable of performing AOM coupled to denitrification (Ettwig et al., 2010; Haroon et al., 2013). Although SO_4^{2-}, NO_3^-, and NO_2^- are relatively lacking in most anoxic freshwater sediments (Smemo and Yavitt, 2007), the ANME-2d cluster has been found in low-SO_4^{2-}, Fe-rich freshwater sediments (Weber et al., 2017), and populations of NC10 bacteria have been detected, in low abundance, in both hypoxic and anoxic lake sediments (Beck et al., 2013; Deutzmann et al., 2014). Interestingly, aerobic methane-oxidizing bacteria (MOB) belonging to gammaproteobacterial MOB (Biderre-Petit et al., 2011; Blees et al., 2014; Kalyuzhnaya et al., 2013; Oswald et al., 2016) have been found in anoxic lake sediments as well (Deutzmann et al., 2014). Furthermore, some gammaproteobacterial MOB were recently found to be active in anoxic zones of the water column and in sediments of lakes (Oswald et al., 2016; Martinez-Cruz et al., 2017), challenging the dogmatic "strictly aerobic" view of MOB (Chistoserdova, 2015; Kalyuzhnaya et al., 2013).

Here, we studied the occurrence and ubiquity of AOM across a global gradient of lake types and conducted an in-depth study of AOM magnitude and mechanisms in sediments from three lakes, two Alaskan and one German. AOM activity and specific mechanisms were addressed using physical and chemical characterization of sediments, development of a diffusion-reaction model, and qPCR for known methanotrophs. Our results indicate that AOM in lake sediments is a globally widespread process that could significantly constrain atmopheric CH_4 emissions.

2. Materials and methods

2.1. Experimental protocol

We demonstrated AOM activity across a climatic, trophic, and physicochemical gradient of 14 tropical, temperate, and arctic lakes (Table S1) using incubations of surface sediments. Then, a detailed CH_4 cycling study was done in 25 cm deep sediment cores from three of the study lakes where AOM was identified. These incubation experiments included determination of dissolved ions previously identified as possible electron acceptors for AOM (SO_4^{2-}, NO_2^-, NO_3^-, Fe, and Mn (Zehnder and Brock, 1980; Deutzmann et al., 2014; Nordi et al., 2013; Sivan et al., 2011)), determination of headspace and dissolved CH_4 and CO_2 concentrations, and measurements of $^{13}\delta C$ for both gases. The results were then validated with a diffusion-reaction mathematical model that predicts the dissolved CH_4 profile along the 25 cm sediment cores based on the measured AOM and CH_4 production rates. The experimental approach is illustrated in detail in Fig. S1.

2.2. Sample collection

In each of our 14 study lakes (Table S1), we collected two surface lake sediment samples at the center of each lake with a dredge (Ekman, AMS Inc., USA) with the goal of estimating microbial activity within the most active and representative sediment layer (Marotta et al., 2014). The Ekman dredge collected the top 15–20 cm of the sediments. The top 5 cm was taken from each sample, immediately placed in a Ziploc bag, and carefully sealed under water to avoid further contact with air. To estimate AOM activity in vertical sediment profiles, three of the fourteen lakes were further investigated: Doughnut Lake, an interior Alaska oligotrophic, partially drained lake; Vault Lake, an interior Alaska thermokarst lake formed in yedoma permafrost with an actively expanding thaw bulb; and Dagow Lake, a temperate eutrophic lake located in northern Brandenburg, Germany. Doughnut and Vault

Lakes are described in detail by Sepulveda-Jauregui et al. (2015), while further details for Dagow Lake can be found in the study by Casper (1996). In each of these three lakes, three 25 cm surface sediment cores were collected using 6.6 cm diameter polycarbonate tubes and a piston hammer corer (Aquatic Research Instruments, USA) or a gravity corer (Uwitec, Austria) from the approximate center of each lake in March and April 2013 (Alaska) and in June 2014 (Germany). Each core was subdivided into six sections (0–2.5, 2.5–5, 5–10, 10–15, 15–20, and 20–25 cm) for further analysis and experimentation.

2.3. Physical and chemical analysis

Dissolved CH_4 and CO_2 concentrations in each section of the Doughnut, Vault, and Dagow Lake cores were measured in triplicate following He et al. (2012). Immediately after collection, 5 mL of wet sediment was gently transferred into 20 mL serum vials containing 5 mL of CH_4 and CO_2-free water, which then were sealed with rubber stoppers and frozen until headspace CH_4 and CO_2 analysis (performed within 72 h of sampling) using a gas chromatograph equipped with both a thermal conductivity detector and a flame ionization detector (Shimadzu GC-2014, Japan). The $^{13}CH_4$ and $^{13}CO_2$ isotopic ratios in each sediment section were determined with a Picarro G2201-i Analyzer (Picarro Inc., USA) for the Alaskan lakes and a gas chromatograph coupled to a combustion isotope ratio mass spectrometer system (GC-C-IRMS, Thermoquest, USA; Conrad et al., 2009) for Dagow Lake. Given the presence of an active thaw bulb under Vault Lake, we also collected bubbles to determine if the released CH_4 was produced from fresh organic matter at the sediment surface or ancient organic matter coming from within the thaw bulb itself. The analysis of $^{13}CH_4$ isotopic ratios of the bubbles is described in the supplementary information (Section S1.3). Pore water from each section of the cores of the three lakes was sampled by extracting ~30 g aliquots, centrifuging them at 415 G for 15 min, and then passing the supernatant through a 0.45 μm filter (Whatman, OE67, USA). The pore water was then analyzed for nitrate (NO_3^-) and nitrite (NO_2^-) according to standard methods (APHA, 1989) and for total iron (Fe) and total manganese (Mn) according to standard colorimetric methods (APHA, 1989; Casper, 1996). Sulfate (SO_4^{2-}) was determined with a Dionex Ion Chromatogram system (detection limit 5 μM; Dionex Inc., ICS 1500 in Alaska, USA, and ICS 1000 in Stechlin, Germany), and dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) with an Aurora TOC Analyzer (Aurora TOC 1030W, O.I. Analytical, USA) for Doughnut and Vault Lakes and Shimadzu TOC elemental Analyzer (TOC-V_{CSH}, Shimadzu, Japan) for Dagow Lake. Sediment dry weight and loss on ignition (LOI) were determined via APHA methods (1989) from ~5 g aliquots of each core section prior to centrifugation.

2.4. Incubation tests

The fourteen lake sediments used for preliminary screening were incubated as explained in the supplementary information (section S1.2). For the detailed core studies of the three lakes, methanogenic, aerobic methanotrophic, and AOM tests were performed with triplicate sections of the sediment cores (0–2.5, 2.5–5, 5–10, 10–15, 15–20, and 20–25 cm). Slurries of each section containing 60% fresh sediment and 40% anoxic deionized water were prepared under a continuous flow of ultra-high purity N_2 (99.999%, AirGas, USA) to maintain anoxic conditions. Then, 30 mL of each slurry was transferred to a 50 mL serum bottle and flushed continuously with N_2. Six bottles, designated for either AOM and methanogenesis determination, were maintained under anoxic conditions via continued flushing with N_2 for five minutes, whereas three bottles, designated for aerobic methanotrophy determination, were flushed with synthetic air (20% O_2, 80% N_2; AirGas, USA) for five minutes. Finally, all bottles were sealed with blue butyl rubber stoppers (Bellco, USA) and aluminum crimp caps. In AOM and methanogenic replicates, Na_2S was added to reduce the media (0.05 g l^{-1} (Moran et al., 2008)). All AOM vials were spiked with 2 mL of $^{13}CH_4$ (99 atom % ^{13}C, Sigma-Aldrich), whereas all vials for aerobic methanotrophy were spiked with 2 mL of CH_4 99%. Five sterilized controls (121 °C, 20 min) for each treatment and each core sample were also prepared. These control incubations received the same treatment as their corresponding non-sterilized samples and were maintained at the same incubation temperature. Control vials containing water and resazurin as an oxidation–reduction indicator were prepared using the same method to confirm anaerobicity. Incubations were carried out at 4 ± 2 °C (Alaska) and 8 ± 3 °C (Germany), representing the annual mean sediment temperature per lake. Headspace concentrations of CH_4, CO_2, and O_2 were monitored as described above at 3-, 15-, and 30-day intervals for aerobic methanotrophic potential (AMP) rate, net methanogenic production rate (NMPR), and AOMR, respectively. Each AOM incubation sample was also monitored for isotopic $^{13}CH_4$ and $^{13}CO_2$ as previously described. The concentration of $^{13}CH_4$ was determined per eq. (1):

$$\delta^{13}C - CH_4 = \left[\frac{\left(\frac{13CH4_{sample}}{12CH4sample}\right)}{0.0112} - 1\right] \times 1000\ ‰ \quad (1)$$

$\delta^{13}C$–CH_4 is the isotopic signature measured at each sampling time; and $^{12}CH_{4\ sample}$ is defined as $^{Total}CH_{4\ sample} - {}^{13}CH_{4\ sample}$, where $^{Total}CH_{4\ sample}$ corresponds to the CH_4 concentration determined by gas chromatography. The ^{13}C:^{12}C of Pee Dee Belemnite is 0.0112.

NMPR was determined from a linear fit of the headspace CH_4 concentration (total CH_4, i.e., $^{12}CH_4 + {}^{13}CH_4$) change over time and corresponds to the total or gross CH_4 production rate minus AOMR. As during the AOM incubations, the ^{12}C-CH_4 produced by methanogenesis and then oxidized was not considered (i.e., AOMR was conservatively estimated). AMP rates were determined from the linear decrease in headspace CH_4 concentrations in methanotrophic vials, whereas AOMRs were calculated from the linear decrease in $^{13}CH_4$ concentration over time. As with AOM incubations, the AOMR was conservatively estimated and the ^{12}C-CH_4 being produced by methanogenesis and then oxidized was assumed to be unimportant.

2.5. Diffusion-reaction model

The results obtained from incubations and the dissolved CH_4 concentrations were used to feed a diffusion-reaction mathematical model. We used a previously described standard diffusion-reaction model (Chuang et al., 2016; Trolle et al., 2010) to illustrate biogeochemical sediment profiles (eq. (2)), where C is the CH_4 concentration, D is the CH_4 effective diffusion coefficient, and z is the depth.

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial z^2}\right) + NMPR \quad (2)$$

Our model did not consider burial of CH_4 (i.e., we assumed CH_4 was produced endogenously only). We also assumed steady-state conditions (i.e., concentration and rates did not change over time) and used C at the water/sediment interface and at the deepest segment of the core as boundary conditions. Based on the experimental NMPR, the model was fitted to the experimental CH_4

concentration data, with least square error minimization performed in R (v 3.3.0, R Core Team, 2013. R Foundation for Statistical Computing, Austria) using the CH_4 diffusion coefficient as the sole adjustment parameter.

2.6. DNA extraction and qPCR analyses

To confirm the presence of gammaproteobacterial MOB potentially involved in AOM in sediments under anoxic conditions, which were previously identified in Vault Lake (Martinez-Cruz et al., 2017), *pmoA* genes of bacteria-like *Ca.* M. oxyfera (pmoA-MB) and *pmoA* genes of aerobic methanotrophs (pmoA-AM) in Vault and Doughnut Lakes were quantified. From each AOM incubation subsample, DNA was extracted in duplicate from 0.25 ± 0.08 g of wet sediment, before and after incubation, using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, USA). qPCR was conducted in triplicate using an ABI 7500 Sequence Detection System. Reactions (10 μL) contained 5 μL 2X SYBR® FAST qPCR Master Mix (KAPA Biosystems, USA), 0.2 μL forward primer (200 nM final), 0.2 μL reverse primer (200 nM final), and 0.2 μL ROX Low passive reference dye (ThermoFisher; USA), 3.4 μL RT-PCR grade water, and 1 μL template DNA. pmoA-MB genes were amplified using the primer pair HP3F1 and HP3R1 (Han and Gu, 2013) and pmoA-AM genes using the primer pair A189F and A682R (Martineau et al., 2010). Standards used were custom gBlocks® Gene Fragments (Integrated DNA Technologies, USA). Thermal cycler conditions for pmoA-AM qPCR were as follows: an initial stage at 50 °C for 2 min; denaturation at 95 °C for 3 min; and 40 cycles of 95 °C for 10 s, 58 °C for 30 s, and 72 °C for 30 s. Thermal cycler conditions for pmoA-MB qPCR were as follows: an initial stage of denaturation at 95 °C for 10 min and 40 cycles of 95 °C for 15 s, 54 °C for 45 s, and 72 °C for 15 s. Cycling was followed by a dissociation stage. After qPCR, dissociation curves were checked for assay specificity. Standard curves were checked for PCR efficiency and for R^2 above 0.98. Detection limits were below 10^2 copies. In triplicate reactions, up to one of the three data points were manually excluded from analysis if determined to be an outlier. Data are reported as gene copy numbers per cm^3 of fresh sediment.

2.7. Statistical analysis

Statistical analyses were carried out on the AOMR, NMPR, and gene copy number for the Vault and Doughnut Lake samples. For all data, normality was assessed by the Shapiro–Wilk test, as most of the data was non-normally distributed. Significant differences among variables were determined using the Mann–Whitney U (MWU) comparison test ($p < 0.05$) when comparing only two variables and the Kruskal–Wallis (KW) multiple comparison test ($p < 0.05$) when comparing more than two variables. All statistical analyses and linear regressions were conducted using the NCSS 11 Statistical Analysis System software (Number Cruncher Statistical Systems).

3. Results and discussion

3.1. Incubation tests

All 14 lakes showed either low dissolved oxygen (<1.5 mg L^{-1}) or anoxic conditions at the bottom of the water column, suggesting anoxia in the surface lake sediments. The AOM activity from the 14 selected lakes, identified by measuring labeled carbon dioxide ($^{13}CO_2$) production, was significantly higher than that for the sterile controls in all but one temperate lake (#7; Table S1). This provides evidence for widespread AOM in lake sediments independent of lake trophic status or climate regime and suggests either a range of potential electron acceptors or a single ubiquitous electron acceptor not linked to lake productivity and nutrient dynamics.

Our detailed investigation of AOM across vertical sediment profiles of three lakes ((Doughnut (oligotrophic, arctic), Vault (dystrophic/thermokarstic, arctic), and Dagow (eutrophic, temperate) Lakes) demonstrated significant AOM and CH_4 production in all three lakes, at all sediment depths (Fig. 2). The AOMR, determined from $^{13}CH_4$ uptake, ranged from 3.30 ± 0.36 nmol cm^{-3} d^{-1} at the bottom (20–25 cm) of the Doughnut Lake core to 16.30 ± 1.38 nmol cm^{-3} d^{-1} at the top (0–2.5 cm) of the Vault Lake core. AOMR for all three lakes and at all depths ranged from 0.6 to 20.9 nmol cm^{-3} d^{-1}, with a median of 7.8 nmol cm^{-3} d^{-1}. These values are similar to the range of AOMR in lake sediments previously reported in other studies, with or without electron acceptor amendments (Fig. 1a; 0–180.0 nmol cm^{-3} d^{-1} amended and 0–43.0 nmol cm^{-3} d^{-1} unamended), although those studies had a lower median of 3.5 nmol cm^{-3} d^{-1} (Fig. 1a).

The NMPR, which corresponds to the gross CH_4 production rate minus AOMR, ranged from 1.10 ± 0.26 at the bottom (20–25 cm) of the Vault Lake core to 83.18 ± 11.85 nmol cm^{-3} d^{-1} at the top (0–2.5 cm) of Dagow Lake core (Fig. 2). In the sediments of Dagow Lake, which had the highest trophic index and greater DOC (Table S3), both NMPR and AOMR were significantly higher ($p < 0.05$) than those observed in the other two lakes. This supports previous studies that showed that microbial communities can efficiently use a broader range of substrates in lake sediments with higher carbon availability and trophic index (Torres et al., 2011), thereby fueling CH_4 production (Casper, 1992) and enhancing CH_4 cycling (Blazewicz et al., 2012). However, Dagow Lake sediments were incubated at higher temperature (8 °C) than Alaska lake sediments (4°), which is also known to enhance methanogenesis, especially in eutrophic lakes (Sepulveda-Jauregui et al., 2018).

The percentage of gross CH_4 production oxidized anaerobically ranged from 12% in the bottom of the Doughnut Lake core to 87% in the bottom of the Vault Lake core (Fig. 2). The average oxidation percentage in the sediment cores of the three lakes was 35 ± 26% ($n = 18$), with a 95% confidence interval of 30%–41%. Our study was limited to three lakes and did not consider temporal variation or seasonal differences, which a full budget approach would require. However, from the meta-analysis presented in Fig. 1, a balance between AOMR from unamended incubations and CH_4 production rates previously reported gives a percentage of oxidation value that is relatively close (i.e., ranging from 0 to 48%, with a median of 18%, which is within the lower range in our experiments). Altogether, our results combined with literature data ($n = 93$) give a percent of gross methane production oxidized in surface lake sediments, a reduction in potential methane emissions, of 29%–34% (95% confidence interval), which reinforce suggestions that AOM in lake sediments is an important constraint on the CH_4 release from lake sediments. This result implies that in the littoral region of lakes or shallow lakes where ebullition is relatively more important and CH_4 bubbles can evade aerobic methanotrophy (Natchimuthu et al., 2016), AOM could be the primary constraint on the CH_4 release from lake sediments.

In our three primary study lakes, the highest NMPR and AOMR were observed in the top core sections, whereas the percentage of CH_4 oxidized by AOM was the highest in the top section of Doughnut Lake and in the deeper sections of Vault and Dagow Lakes. Therefore, in Vault and Dagow Lakes, the high AOMR observed in the top section of the cores was offset by high CH_4 production, resulting in relatively low CH_4 oxidation percentages. The pattern of greater NMPR in the upper sediment layer of Vault and Dagow Lakes is similar to results reported by Heslop et al. (2015), where the CH_4 production rate was greatest in surface sediments of Vault Lake. Doughnut Lake did not show the same

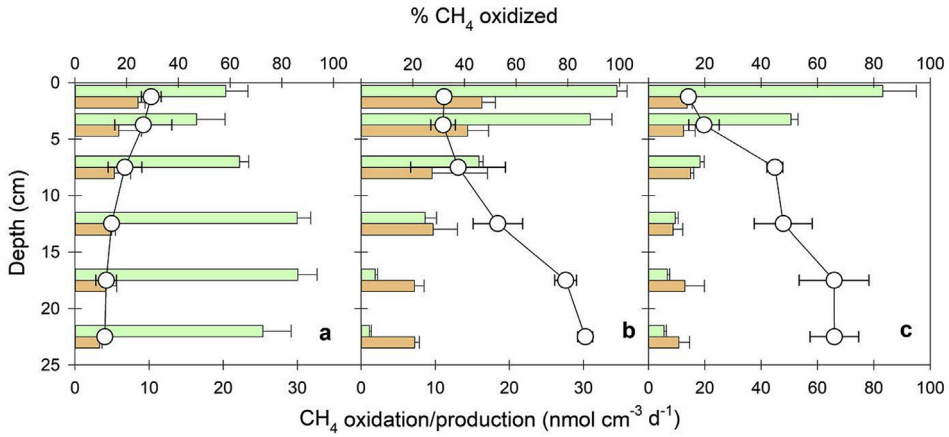

Fig. 2. CH_4 oxidation rate (AOMR; orange bars), net CH_4 production rate (NMPR; green bars), and the percentage of CH_4 that is oxidized by AOM (open circles). Percentage of AOM was determined from AOMR and the total CH_4 production rate [(AOMR*100)/TMPR]; (a) Doughnut Lake; (b) Vault Lake; (c) Dagow Lake. Error bars represent one standard deviation for incubation replicates. Please note the different scale in (c). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

trend, as relatively high NMPR was still observed in the deepest core section. The relatively high NMPR we observed coincided with lower DOC concentrations compared to those in Vault and Dagow Lakes (Table S3). CH_4 production in freshwater sediments is often limited by the availability of labile organic matter (Hoyos-Santillan et al., 2016; Sobek et al., 2012), which represents only a fraction of total DOC. It is possible that the labile fraction of DOC was significantly greater in Doughnut Lake than in the other lakes despite no observed differences in sediment LOI across sites (Table S3).

3.2. Diffusion-reaction model

To assess how our observed potential rates matched prevailing sediment conditions, the production/oxidation rates presented in Fig. 2 were used as inputs in a diffusion-reaction model (eq. (2)). The model predicted dissolved CH_4 concentration in the cores, which could in turn be compared to experimental data. The modeled dissolved CH_4 concentration matched well with the field data (Fig. 3), indicating that rates determined from laboratory incubations corresponded to those present in sediments. We interpret this as further support for our findings that lake sediment AOM is an important constraint on global greenhouse gas emissions. Best fittings were obtained with effective diffusion coefficients of 4.5×10^{-5}, 6.5×10^{-5}, and 9.0×10^{-5} $cm^2\ s^{-1}$, for Vault, Doughnut, and Dagow Lakes, respectively. These effective diffusion coefficients are about one order above previously reported values (Iversen and Jorgensen, 1993; Gruca-Rokosz and Tomaszek, 2015), but as indicated by Flury et al. (2015), the presence of minor amounts of gas can increase the diffusion of gases by several orders of magnitude. Likewise, bioturbation impacts of benthic animals may also increase gas transport within sediments significantly (Baranov et al., 2016).

3.3. Isotopic signatures

The isotopic signatures of CH_4 and CO_2 along the sediment cores are shown in Fig. 4. The lowest $\delta^{13}C$-CH_4 values were observed at depths greater than 5 cm in the sediment cores, with minimum (depleted) values of −66.7‰, −61.4‰ and −61.3‰ for Doughnut, Vault, and Dagow Lakes, respectively. These values are consistent with isotopic signatures previously reported for lake sediments (e.g., Conrad et al., 2009; Whiticar, 1999), indicating microbial CH_4 production. The highest $\delta^{13}C$-CH_4 values −36.9‰, −49.8‰ and −59.7‰ for Doughnut, Vault, and Dagow Lakes, respectively, which were all measured in the first 2.5 cm of the sediment cores. Doughnut and Vault Lake isotopic signatures may indicate a ^{13}C enrichment due to CH_4 oxidation in the upper sediment layers, as previously reported (Schubert et al., 2011). Indeed, in Doughnut and Vault Lakes, the enriched carbon ^{13}C-CH_4 observed in the top section can be explained by the higher observed AOMR (Fig. 2),

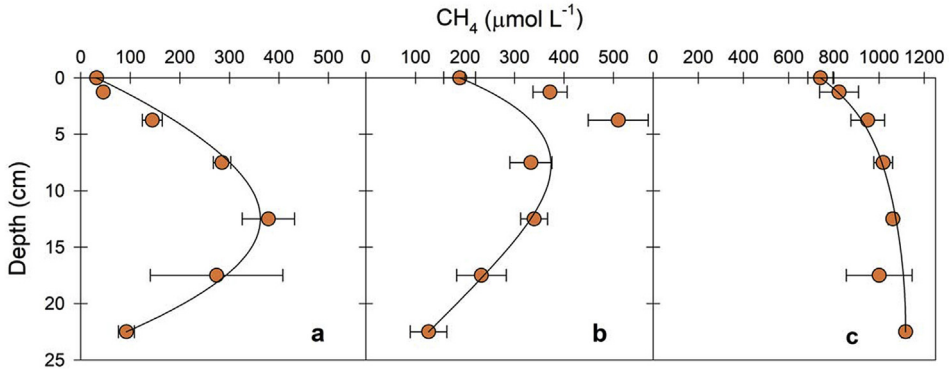

Fig. 3. Diffusion-reaction model fitting (–) and dissolved CH_4 concentration (●) measured in sediment cores in (a) Doughnut, (b) Vault, and (c) Dagow Lakes. Error bars represent one standard deviation. Correlation coefficients are 0.986, 0.782, and 0.956 for a, b, and c, respectively.

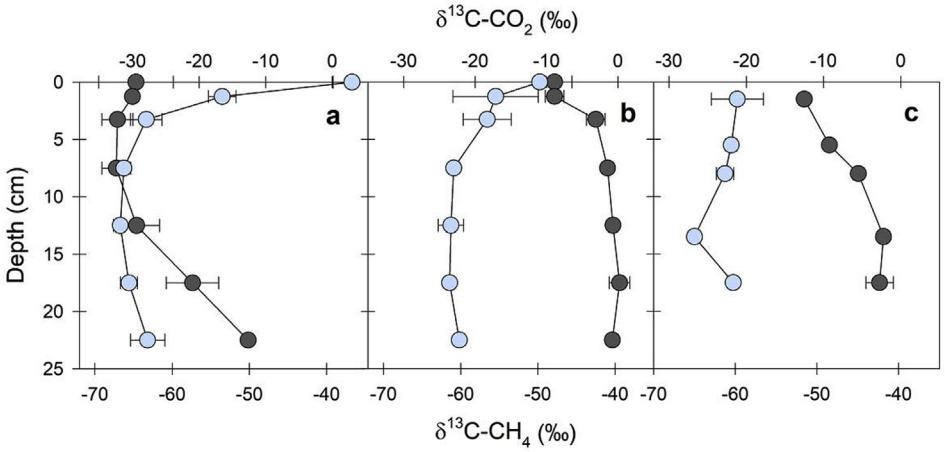

Fig. 4. Profiles of δ^{13}C-CH_4 (●) and δ^{13}C-CO_2 (●) for Doughnut (a), Vault (b), and Dagow (c; Conrad et al., 2009) Lakes. Error bars show one standard deviation.

whereas in Dagow Lake, the relatively small difference in the isotopic signatures (<2‰) along the sediment core may be driven by the relatively uniform AOMR profile (Fig. 2). In Doughnut Lake, we observed a correlation between dissolved CH_4 concentration and δ^{13}C-CH_4 (Fig. S2), a relationship that has been previously reported as evidence of CH_4 oxidation (Grant and Whiticar, 2002). This correlation was not observed in Vault and Dagow Lakes, which suggests different carbon cycling processes among the lake cores. In Vault Lake, CH_4 bubbles released from the base of the thaw bulb (>5 m below the sediment surface) and those collected at the lake surface had an isotopic value of -72.7 ± 4.3‰ ($n = 4$) δ^{13}C-CH_4. Presence of this relatively depleted CH_4 suggests that ebullition of bubbles originating from greater depths in thaw bulbs are less influenced by AOM than by CH_4 produced in surface sediments. The δ^{13}C-CO_2 profiles in the sediment cores of the three studied lakes exhibited a distinguishable symmetry between δ^{13}C-CH_4 and δ^{13}C-CO_2, with relatively light CO_2 combined with heavy CH_4 in the uppermost sediments, which has been suggested as evidence for AOM (Schubert et al., 2011; Segarra et al., 2015). The δ^{13}C-CO_2 profiles agree with CO_2 production by acetoclastic methanogenesis or AOM in the upper sections of the sediments and CO_2 reduction, potentially by hydrogenotrophic methanogenesis, in deeper sediment layers (Conrad et al., 2009). The isotopic evidence together with the dissolved gases indicate very active CH_4 cycling along the 25 cm of sediment cores of the three studied lakes, with possibly higher AOM activity in the top sediment layers. The AOM incubation tests and the diffusion-reaction model have confirmed the latter.

3.4. Potential electron acceptors

To identify AOM electron acceptors, we analyzed each subsection of the three cores for dissolved NO_2^-, NO_3^-, and SO_4^{2-} and total Fe and Mn concentrations. The mean concentration of NO_3^- in the lake sediment ranged from 1.4 to 10.5 μmol L^{-1} (Fig. 5), whereas the concentration of NO_2^- was <1.5 μmol L^{-1}. These concentrations were below or at the lower range of concentrations reported in sediments where AOM is coupled to denitrification (Deutzmann et al., 2014; Ettwig et al., 2009), and we observed no correlation between dissolved CH_4 and NO_3^- or NO_2^- concentration. AOM linked to denitrification in any part of our study is therefore unlikely. Total Fe and Mn were detected in lake sediments, at concentrations ranging from 176 to 872 μmol g_{dw}^{-1} and from 4.1 to 24.5 μmol L^{-1}, respectively (Fig. 5). Although the oxidation state of neither element was quantified, and we cannot evaluate their particular role in AOM, we also cannot exclude their role as AOM electron acceptors in these lake systems. Dissolved SO_4^{2-} concentrations in the three lake sediments ranged from 0.3 to 36.9 μmol L^{-1} (Fig. 5), with the smallest concentrations observed in Dagow Lake and the greatest in Vault Lake. Weber et al. (2017) demonstrated AOM coupled to SO_4^{2-} reduction at SO_4^{2-} levels below 100 μmol L^{-1}, suggesting SO_4^{2-} was a potential electron acceptor in our study lakes. However, contrary to previous studies that have implicated SO_4^{2-} as the AOM electron acceptor (Beal et al., 2011; Schubert et al., 2011), we observed no negative relationship between SO_4^{2-} and dissolved CH_4 concentrations for Vault and Doughnut Lakes (Fig. 5a and b). The SO_4^{2-} concentration in Dagow Lake did illustrate a negative relationship (Fig. 5c). Nevertheless, the SO_4^{2-} concentration reached values below 1 μmol L^{-1}, an insufficient amount of SO_4^{2-} to account for the observed AOM activity. Overall, our electron acceptor investigation was inconclusive. Previous studies have demonstrated complex interactions between metals and SO_4^{2-} or humic substances that might serve as electron acceptors (Egger et al., 2015; He et al., 2018; Nordi et al., 2013; Sivan et al., 2011; Smemo and Yavitt, 2007; Valenzuela et al., 2017). It is possible that a combination of electron acceptors and pathways could be naturally occurring in our study lakes and explain why we found no correlation between AOMR and lake trophic state or climate in our broader 14 lake study.

3.5. Potential aerobic methanotrophy

Sediment samples also were tested for potential aerobic methanotrophy in vials flushed with synthetic air and spiked with CH_4. The aerobic methanotrophic potential (AMP) ranged from 123.2 nmol cm^{-3} d^{-1} in the mid-section (5–10 cm) of the Dagow Lake core to 323.2 nmol cm^{-3} d^{-1} at the top (0–2.5 cm) of the Doughnut Lake core (Fig. S3). These potential rates are within the reported ranges for similar anoxic lake sediments and freshwater ecosystems (Chan et al., 2005; Deutzmann and Schink, 2011; He et al., 2012). Interestingly, we found a linear relation between AOMR and AMP (Fig. S4), which suggests that aerobic methanotrophs might be involved directly or indirectly in AOM, despite the anoxic conditions prevailing in the cores. The latter agrees with previous reports, which have indicated possible involvement of aerobic methanotrophs in AOM (Biderre-Petit et al., 2011; Blees et al., 2014; Chistoserdova, 2015; Kojima et al., 2012; Martinez-Cruz et al., 2017; Oswald et al., 2016), and there is clear evidence for *Candidatus* Methylomirabilis oxyfera bacteria performing intra-aerobic CH_4 oxidation in anoxic environments using NO_2^- as an

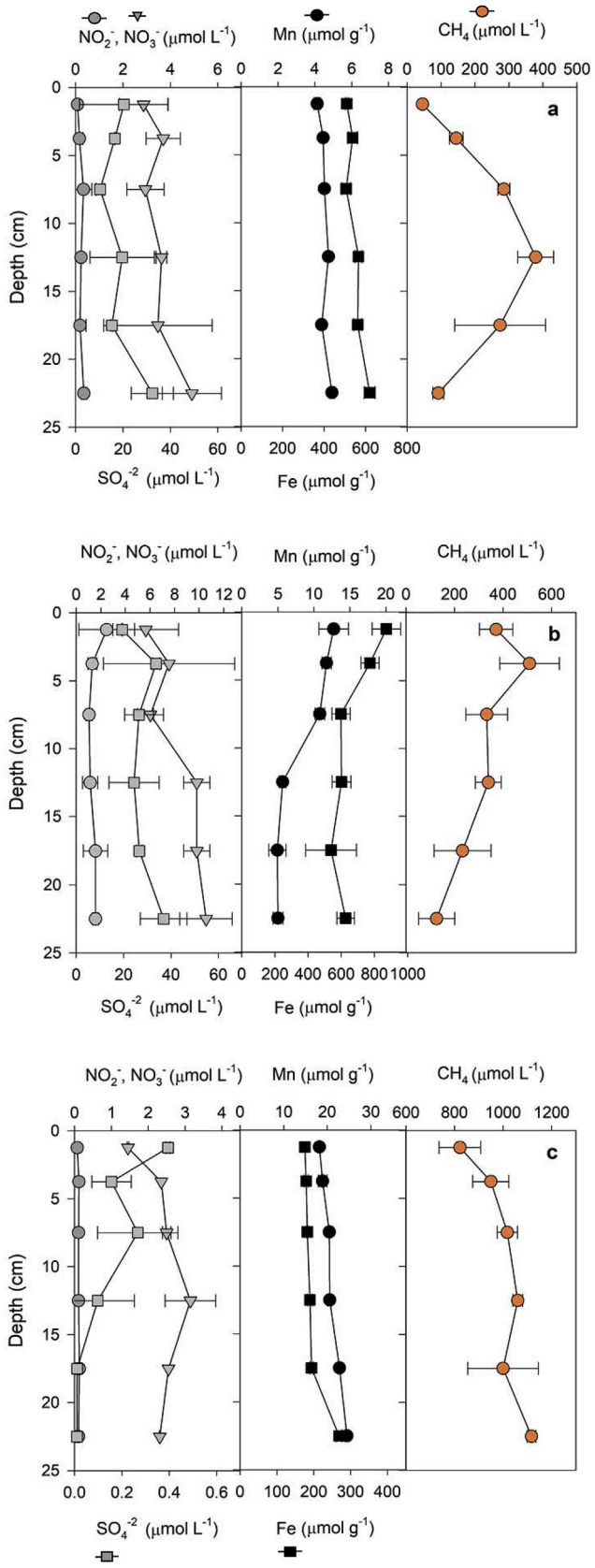

Fig. 5. Dissolved concentration of potential AOM electron acceptors (NO_3^-, NO_2^-, SO_4^{2-}, Fe, and Mn) and CH_4 measured in sediment cores from (a) Doughnut, (b) Vault, and (c) Dagow Lakes. Error bars represent one standard deviation.

electron acceptor (Ettwig et al., 2010). Therefore, we evaluated potential involvement of aerobic methanotrophs in AOM in our lake sediments; *pmoA* genes of both *Ca.* M. oxyfera bacteria-like (pmoA-MB) and aerobic methanotrophs (pmoA-AM) were quantified via qPCR for each section of the sediment cores from Doughnut and Vault Lakes (Fig. S5). The abundance of pmoA-MB ranged from 3.5×10^4 to 1.3×10^5 gene copies cm^{-3} of fresh sediment in Doughnut Lake and from 3.4×10^4 to 1.2×10^5 gene copies cm^{-3} of fresh sediment in Vault Lake. The abundance of pmoA-AM ranged from 4.2×10^6 to 2.5×10^7 and from 3.5×10^5 to 7.2×10^6 gene copies cm^{-3} of fresh sediment in Doughnut and Vault Lakes, respectively. No correlation was found between AOMR and the number of gene copies of both pmoA-AM and pmoA-MB. Moreover, for both groups of microorganisms, no significant differences were found between most of the initial and final gene concentrations (i.e., quantified at the beginning and the end of the incubations). However, a significant difference was observed between pmoA-MB and pmoA-AM ($p < 0.05$), as pmoA-AM was relatively more abundant, with up to three orders of magnitude more gene copies than pmoA-MB had. Given that NO_3^- and NO_2^- concentrations are likely too small to support AOM coupled to denitrification, the low abundance of pmoA-MB in comparison to pmoA-AM is understandable and suggests that *Ca.* M. oxyfera bacteria-like did not contribute substantially to CH_4 oxidation in these lake sediments. Previous lake studies have also reported a high relative abundance of aerobic methanotrophs under anoxic (Bar-Or et al., 2017; Chistoserdova, 2015; Martinez-Cruz et al., 2017; Oswald et al., 2016) or hypoxic (Blees et al., 2014; Kalyuzhnaya et al., 2013; Kits et al., 2015) conditions. Furthermore, the co-occurrence of aerobic and anaerobic CH_4 oxidizers in anoxic lake sediments and water was reported previously (Deutzmann et al., 2014; Oswald et al., 2016). In anoxic environments, aerobic methanotrophs are likely i) dormant for extended periods of time with the ability to resume their methanotrophic activity when the environment is conducive for such (Roslev and King, 1994), ii) able to survive prolonged starvation probably by fermenting endogenous substrates (Roslev and King, 1995), and iii) able to utilize alternative electron acceptors such as Fe, Mn, or NO_3^- (Ettwig et al., 2010). The latter has been mainly attributed to "aerobic" gammaproteobacterial methane oxidizers (Martinez-Cruz et al., 2017; Oswald et al., 2016). However, the direct links between gammaproteobacterial methane oxidizers and the reduction of alternative electron acceptors remain uncertain. Coupling of aerobic methanotrophy to oxygen production or cryptic oxygen cycling (Chistoserdova and Kalyuzhnaya, 2018; Garcia-Robledo et al., 2017) is an additional hypothesis. However, that coupling has thus far been linked to an active photosynthetic community fueling aerobic methanotrophy in anoxic lake zones (Milucka et al., 2015), which is unlikely to occur in our relatively-deep-lake sediments. Thus, the relatively high abundance of pmoA-AM may hint at the still unknown process carried out by aerobic methanotrophs, which is similar to the already known process carried out by anaerobic CH_4 oxidizers. We acknowledge that by targeting ANME groups, a more complete picture of potential AOM organisms could have been obtained. Thus, further lake sediment AOM studies should focus on elucidating the relationship between aerobic methanotrophs and ANME groups.

4. Conclusions

Overall, our findings greatly reduce the uncertainty related to the magnitude and spatial ubiquity of AOM in lake sediments. Identifying AOM in thirteen out of fourteen lakes across latitudinal and trophic gradients, together with the determination of AOM rates in three sediment cores and a further cross-validation of those rates with a diffusion-reaction model, provides rigorous support for

significant microbial CH_4 sinks in lake sediments. Combined with those in previous reports, our results suggest that AOM may represent a sink of one third of the total CH_4 produced in lake sediments. The remainder CH_4 then migrates through the water column where it is subject to aerobic oxidation (Martinez-Cruz et al., 2015). Thus, a significant fraction of the CH_4 produced in lakes is recycled internally, via aerobic or anaerobic oxidation, whereas only a reduced portion is emitted to the atmosphere. We acknowledge that our results are still fractional, and that further research is needed to constrain better the global magnitude of AOM, but this process might be of the same order of magnitude as the global CH_4 emission from lakes, which is estimated at 72 Tg y^{-1} (Bastviken et al., 2011). In addition, based on the evidence presented in this study, it is not possible to attribute the AOM process coupled to a specific electron acceptor or microbial group, which continues to frustrate our understanding of the process.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2018.07.053.

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