

Are elusive anaerobic pathways key methane sinks in eutrophic lakes and reservoirs?

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Abstract Collectively, freshwaters constitute a significant source of methane to the atmosphere, and both methane production and methane oxidation can strongly influence net emissions. Anaerobic methane oxidation (AOM) is recognized as a strong regulator of marine methane emissions and appreciation of AOM's importance in freshwater is growing. In spite of this renewed interest, recent work and reactive-transport modeling results we present in this paper point to

unresolved pathways for AOM. Comparison of recent observations from a eutrophic reservoir, Lacamas Lake, with predictions of a 1D steady-state model of water column methane dynamics indicates that high rates of methane oxidation measured via bottle assays cannot be explained with conventional electron acceptors (O_2 , NO_2^- , NO_3^- , SO_4^{2-} , Mn^{4+} , and Fe^{3+}). Reactive-transport modeling suggests that solute oxidant concentrations at the thermocline would have to be around 10 times higher than observed to explain the measured methane consumption. Organic acids—a major constituent of organic matter—may account for part of this unexplained AOM given their abundance in eutrophic systems, although the details of these pathways remain elusive (e.g., which species are involved, seasonal renewal of reduced species, contribution of particulate versus dissolved phases). We

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point to several observations consistent with organic acid-mediated AOM, both in Lacamas Lake and in other systems. Nevertheless, direct evidence of this pathway is still lacking and testing for this remains an important direction for future work. To this end, we identify several new avenues of research that would help quantify the role of organic acid-mediated AOM relative to other electron acceptors.

Keywords Anaerobic · AQDS · Lake · Methane oxidation · Organic acids · Reactive transport modeling

Introduction

Methane is a potent greenhouse gas with rapidly increasing atmospheric concentrations (Myhre et al. 2013). Collectively, lakes and reservoirs contribute upwards of 90 Tg CH₄ year⁻¹ to the atmosphere (Bastviken et al. 2011), more than 10% of all sources in the global CH₄ budget (Ciais et al. 2013). Still, there is considerable uncertainty associated with modeling and upscaling field-scale CH₄ measurements (Wik et al. 2016). One uncertainty arises from the critically important role of methanotrophs (i.e., methane oxidizers) in attenuating atmospheric CH₄ emissions. In a review of 7 lakes, between 50 and 95% of methane produced in lake sediments was oxidized prior to release (Bastviken et al. 2008).

In the ocean, 70–304 Tg of methane (10–55% of the total global atmospheric CH₄ flux) are oxidized each year (Reeburgh 2007; Ciais et al. 2013). Most (>90%) of this oceanic methane consumption occurs in the absence of oxygen via anaerobic oxidation of methane (AOM, Hinrichs and Boetius 2002; Reeburgh 2007). Marine AOM is generally observed in sediments in a region known as the “sulfate-methane transition zone” where anaerobic methanotrophic archaea are thought to work in concert with a sulfate-reducing “partner” (Knittel and Boetius 2009). Given high concentrations of sulfate (SO₄²⁻) in seawater, sediment-based production of CH₄ rarely outpaces SO₄²⁻ supply from the overlying water column (although exceptions include seeps, vents, and gas-laden tidal flats, Knittel and Boetius 2009). In addition to SO₄²⁻, recent studies have identified a number of alternative electron acceptors that drive AOM across marine and

freshwater ecosystems (nitrate-nitrite-mediated, Ettwig et al. 2010 and Kojima et al. 2014; nitrate, iron, and manganese-mediated, Segarra et al. 2013; iron-mediated, Egger et al. 2015; 9,10-anthraquinone-2,6-disulfonate (AQDS), iron, and humic acid-mediated, Scheller et al. 2016; sulfate, iron, and AQDS mediated, Valenzuela et al. 2017; Table 1). These findings have coincided with work highlighting the potential importance of AOM outside of marine sediments. For example, a recent review estimates that AOM reduces atmospheric CH₄ emissions from wetlands by >50% (Segarra et al. 2015).

Here, we define AOM broadly as all methane oxidation pathways that occur in the absence of oxygen. This includes methane oxidation coupled to SO₄²⁻ reduction by anaerobic methanotrophic archaea (ANME), denitrification by oxygenic bacteria (Ettwig et al. 2010), and other electron acceptors such as iron and manganese oxides (by ANME, gammaproteobacteria and other heretofore unidentified microorganisms, Borrel et al. 2011; Scheller et al. 2016; Oswald et al. 2016).

AOM in lakes and reservoirs

Until recently, relatively little work had been done to characterize AOM in freshwater lakes and reservoirs due, in part, to a widely held assumption that AOM was fueled solely by SO₄²⁻ (Borrel et al. 2011). High concentrations of CH₄ are widely observed to accumulate in the anoxic bottom waters of stratified lakes and reservoirs (Bastviken et al. 2008), but CH₄ oxidation is thought to occur predominantly near the oxycline where micro-aerophilic bacteria can use O₂ as a terminal electron acceptor (TEA, Bles et al. 2014). Still, the potential role of AOM in mediating lake and reservoir CH₄ emissions is starting to gain attention. About 10 years after the discovery of marine AOM, the earliest indications of lentic AOM were reported in bottle incubations from Lake Mendota (Panganiban et al. 1979). Several recent studies have used isotopic, microbiological, and incubation-based evidence as well as reactive-transport modeling to document AOM in the anoxic hypolimnion of lakes and reservoirs (Eller et al. 2005; Pimenov et al. 2010; Schubert et al. 2010; Crowe et al. 2011; Lopes et al. 2011; Bles et al. 2014; Kojima et al. 2014; Saxton et al. 2016; Oswald et al. 2016). Methanotrophs are known to preferentially oxidize

Table 1 Potential methane oxidation pathways

Terminal electron acceptor	Reaction	ΔG° (kJ mol ⁻¹ CH ₄)
Sulfate	$\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$	-33 ^a
AQDS	$\text{CH}_4 + 4\text{AQDS} + 3\text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}^+ + 4\text{AQH}_2\text{DS}$	-41 ^b
Iron oxyhydroxides	$\text{CH}_4 + 8\text{Fe}(\text{OH})_3 + 15\text{H}^+ \rightarrow \text{HCO}_3^- + 8\text{Fe}^{2+} + 21\text{H}_2\text{O}$	-571 ^a
p-Benzoquinone	$\text{CH}_4 + \text{Q} + 2\text{H}_2\text{O} \rightarrow \text{CO}_2 + 4\text{QH}_2$	-731 ^c
Manganese oxides	$\text{CH}_4 + 4\text{MnO}_2 + 7\text{H}^+ \rightarrow \text{HCO}_3^- + 4\text{Mn}^{2+} + 5\text{H}_2\text{O}$	-790 ^a
Nitrate	$\text{CH}_4 + 4\text{NO}_3^- \rightarrow \text{CO}_2 + 4\text{NO}_2 + 2\text{H}_2\text{O}$	-801 ^a
Nitrite	$3\text{CH}_4 + 8\text{NO}_2^- + 8\text{H}^+ \rightarrow 3\text{CO}_2 + 4\text{N}_2 + 10\text{H}_2\text{O}$	-1007 ^a
Oxygen	$\text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O}$	-858 ^d

^a Adjusted from Segarra et al. (2013)

^b Adjusted from Scheller et al. (2016)

^c Calculated based on Uchimiya and Stone (2009), see supplementary materials for detailed calculation

^d Calculated using the CHNOSZ package (Dick 2008)

lighter methane (¹²C isotope), such that regions of high methane oxidation can result in relatively depleted $\delta^{13}\text{C}$ in dissolved inorganic carbon (DIC) and relatively enriched $\delta^{13}\text{C}$ in dissolved methane. Several lake and reservoir studies have reported peaks in methane $\delta^{13}\text{C}$ (Eller et al. 2005; Schubert et al. 2010; Crowe et al. 2011; Itoh et al. 2015; Oswald et al. 2016) and drops in DIC $\delta^{13}\text{C}$ (Crowe et al. 2011) in anoxic regions where microbiological analyses (Eller et al. 2005; Crowe et al. 2011; Kojima et al. 2014; Saxton et al. 2016; Oswald et al. 2016), geochemical evidence (Eller et al. 2005; Saxton et al. 2016) and/or bottle assays (Pimenov et al. 2010; Schubert et al. 2010; Lopes et al. 2011; Saxton et al. 2016; Oswald et al. 2016) suggest the presence of AOM. Together, these studies constitute a growing body of evidence highlighting the potential importance of AOM in eutrophic lakes and reservoirs. In the sections that follow, we draw upon a case study and a synthesis of recent literature to build a case that AOM is important in lakes and reservoirs and that an elusive oxidant(s) may play an important, heretofore underappreciated role in mediating this important biogeochemical process.

Methods

Electron acceptor concentrations and oxidation rate measurements

To examine the balance between methane oxidation and electron acceptor availability, we measured water column temperatures, dissolved methane

concentrations, methane oxidation rates and electron acceptor concentrations along a vertical profile near the deepest site (16.8 m) in a well-characterized lake: Lacamas Lake. Lacamas Lake is a small, monomictic, eutrophic reservoir located in southwest Washington, U.S.A. Sampling was conducted at 4, 5.5, 7, 9, 11, 13, 15, and 17 m depth during mid-fall when the thermocline had begun to deepen but the reservoir had not yet fully mixed (28 Oct 2014). Temperature and oxygen concentrations were measured with a Hach DS5X Sonde. For analysis of NO_3^- , NO_2^- , and SO_4^{2-} , samples were collected with a Van Dorn sampler, filtered (Whatman GF/F 0.45 μm), and stored frozen in acid-washed 30 mL plastic HDPE Nalgene bottles until analyzed. NO_3^- , NO_2^- , and SO_4^{2-} were analyzed on a Westco discrete nutrient analyzer using standard EPA-approved colorimetric methods (method number 353.2 for NO_2^- and NO_3^- , and 4500 for SO_4^{2-} , National Environmental Methods Index, www.nemi.gov). The detection limits were 0.4 $\mu\text{mol L}^{-1}$ for NO_3^- and NO_2^- , and 7.2 $\mu\text{mol L}^{-1}$ for SO_4^{2-} . We estimated the potential role of Fe and Mn oxides as TEAs for methane oxidation by measuring the rate of accumulation of dissolved Fe and Mn in the hypolimnion during four summer stratified seasons (July through early September 2010–2013) using the hypolimnion accumulation method described in Deemer et al. (2011). For analysis of dissolved Mn and Fe, 5 mL aliquots of filtered water samples were acidified with 0.15 mL of concentrated HNO_3 to achieve 3% v/v HNO_3 . Samples were then run on an Agilent 7700 inductively coupled plasma mass

spectrometer (ICP-MS). We consider dissolved Mn and Fe as a proxy for reduced Mn and Fe given that oxidized forms of these metals are quite insoluble and our samples were filtered.

Methane oxidation rates were estimated using difluoromethane (DFM, Sigma Aldrich) as an inhibitor of methane oxidation. 0.5 mL DFM was added to half the 70 mL sample bottles ($n = 4$ for each treatment) at the start of the experiment as in Miller et al. (1998) and Kankaala et al. (2006). All samples were then incubated in the dark for 24 h in water baths within ± 2 °C of lake temperature at the time of collection. Incubations were terminated by addition of ZnCl_2 and a 10 mL ultra-high purity helium headspace was introduced. The headspace was analyzed by a gas chromatograph equipped with an electron capture detector (Hewlett-Packard 5890 Series II-Plus). Headspace methane concentrations were used to calculate original dissolved gas concentrations using the appropriate solubility tables (Weiss and Price 1980). The integrity of vials was confirmed using a Membrane-Inlet Mass Spectrometer (Pfeiffer).

Reactive-transport modeling

Observed profiles for oxygen, methane, and methane oxidation rate were compared with output of a simple numerical model describing the transport and oxidation of methane in Lacamas Lake: the Methane and Oxygen Dynamics in Eutrophic Lakes Model (MODEL² hereafter). MODEL² considers transport

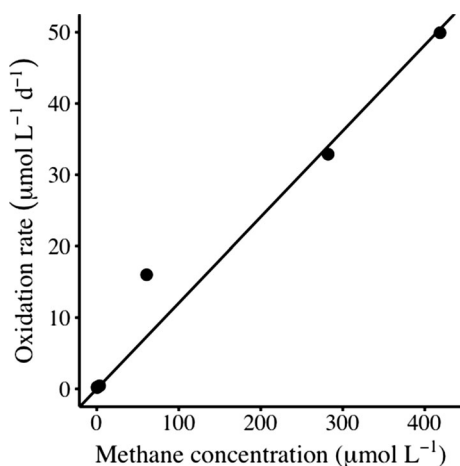


Fig. 1 Methane oxidation rate versus methane concentration. Circles denote observations, while the line shows Eq. (1) fit to these data ($R^2 = 0.97$)

by turbulent mixing and methane oxidation by both aerobic and anaerobic pathways. Consistent with observations (Iversen et al. 1987; Smemo and Yavitt 2011), reaction kinetics for methane oxidation are assumed to be first order with respect to methane concentration (Fig. 1), and methane oxidation rate (R ; $\text{mol L}^{-1} \text{d}^{-1}$) was calculated using the following equation:

$$R = k[\text{CH}_4] \quad (1)$$

where k is the rate constant (d^{-1}) and $[\text{CH}_4]$ is methane concentration (mol L^{-1}). Fitting Eq. 1 to observations using the `lm()` function in the R base package gives a rate constant of 0.12 d^{-1} ($R^2 = 0.97$; Fig. 1), which is similar to previous estimates of aerobic methane oxidation rate constants in lakes (e.g., cf. 0.14 d^{-1} , Lopes et al. 2011). When oxygen is present we assume that methane oxidation progresses aerobically, according to the following reaction:



Upon depletion of oxygen, modeled methane oxidation is attributed to unspecified anaerobic pathways.

We assume that solute (e.g., methane, oxygen) transport is dominated by turbulent mixing, which is quantified using an eddy diffusion coefficient, K_z ($\text{m}^2 \text{s}^{-1}$), that is proportional to the reciprocal of the buoyancy frequency, N^2 (e.g., Katsev et al. 2010):

$$K_z \propto (N^2)^{-1} \quad (2)$$

The constant of proportionality is chosen to reproduce profiles of methane concentration, oxygen concentration, and measured methane oxidation rates. Resulting eddy diffusion coefficients, K_z , are in the range of 10^{-7} – $10^{-5} \text{ m}^2 \text{ s}^{-1}$, which is within the expected range for lakes although at the upper bound for monomictic lakes (e.g., Salas de León et al. 2016). If mixing and, therefore, oxygen supply to the hypolimnion are overestimated in MODEL², then MODEL²-based estimates of AOM are too low.

Within MODEL², the lake is divided into vertical layers of 1 cm in thickness between 5 and 15 m depth. In layer i of the model, oxygen and methane dynamics subject to turbulent diffusive mixing and methane oxidation are modeled using the following volumetric diffusion–reaction equation with the transport term (which incorporates K_z) implemented

in the ReacTran package (Soetaert and Meysman 2012):

$$\frac{d[\text{O}_2]_i}{dt} = -\frac{\Delta_i\{-E \times \Delta[\text{O}_2]\}}{V_i} - 2R_i\delta_{\text{O}_2} = 0 \quad (3)$$

$$\frac{d[\text{CH}_4]_i}{dt} = -\frac{\Delta_i\{-E \times \Delta[\text{CH}_4]\}}{V_i} - R_i = 0 \quad (4)$$

where Δ_i is the difference across the layer, $[\text{O}_2]$ is the oxygen concentration (mol m^{-3}), $[\text{CH}_4]$ is the methane concentration (mol m^{-3}), V_i is the volume of the layer ($A_i \times dz_i$ where A_i is the area and dz_i is the thickness of layer i , respectively; m^3), E is the bulk dispersion coefficient ($K_z \times A_i/dz_i$; $\text{m}^3 \text{d}^{-1}$), R_i is the rate of methane oxidation ($\text{mol m}^{-3} \text{day}^{-1}$), and δ_{O_2} is a switch that forces aerobic methane oxidation to zero when oxygen is depleted. Equations (3) and (4) are solved using the steady.ID() function of the rootSolve package using the stode method (Soetaert and Herman 2009). We define δ_{O_2} as,

$$\delta_{\text{O}_2} = \left(\frac{[\text{O}_2]}{[\text{O}_2] + 3 \times 10^{-6}} \right) \quad (5)$$

We use this expression rather than a binary switch (e.g., $\delta_{\text{O}_2} = \begin{cases} 1, & \text{when } \text{O}_2 > 0 \\ 0, & \text{when } \text{O}_2 = 0 \end{cases}$) because sharp boundaries create problems with the numerical solver. 3×10^{-6} in the denominator is equivalent to a Michaelis–Menten half-saturation coefficient for oxygen of 3 nM, which is the lowest concentration at which microbial growth via aerobic pathways has been observed (Stolper et al. 2010). Data and model code are available for download at <https://github.com/DanielReedOcean/MODEL2>.

Model sensitivity

The model contains two parameters estimated from observations: the first order rate constant for methane oxidation, k , and the eddy diffusion coefficient, K_z . To examine the influence of these parameters on model behavior, we varied the magnitudes of the parameters and reran the baseline scenario described above. The mixing coefficient K_z was varied across 6 orders of magnitude encompassing the typical range of values observed in thermally-stratified lakes (Salas de León et al. 2016) and the observed rate constant (Fig. 1) was varied by $\pm 50\%$. Changes in the mixing coefficient,

K_z , caused all profiles—methane and oxygen concentrations, as well as methane oxidation rate—to deviate from observations, providing confidence in the chosen parameter values (Supplementary Material). While methane and oxygen profiles were largely unaffected by variations in the rate constant, the methane oxidation rate profile departed markedly from observations in the sensitivity analysis (Supplementary Material). A detailed discussion of the sensitivity analysis is included in the Supplementary Material.

Results

MODEL² faithfully reproduces observed oxygen concentration, methane concentration, and methane oxidation rate profiles (Fig. 2). However, comparison of model output and measured oxidation rates reveals that aerobic methane oxidation can account for, at most, just 14% of hypolimnion methane consumption. Other processes almost certainly consume oxygen in the water column (e.g., oxidation of organic matter, Fe^{2+} , Mn^{2+}), so 14% is very likely an overestimate. While these results suggest the occurrence of AOM, traditional anaerobic electron acceptors (e.g. NO_3^- , NO_2^- , and SO_4^{2-} ; Table 2; Fig. 3) are not present in sufficient concentrations to explain the methane oxidation rates observed at Lacamas Lake. The potential for Mn and Fe oxides to fuel AOM also appears to be small given the relatively low rates of reduced Fe and Mn accumulation we observed in the reservoir hypolimnion across a period of 4 years.

NO_3^- , NO_2^- , and SO_4^{2-} are introduced to the model by recasting the oxygen variable as a generic oxidant for methane, G, that represents the sum of all these species. To account for the different stoichiometries of anaerobic methane oxidation, the concentration of G at the upper bound is specified using the measured electron acceptor concentrations at a depth of 4 m (Table 2)—that is, in well-mixed oxygenated surface waters above the thermocline that are the source of oxidants—using the following equation:

$$G_{4\text{m}} = \left[\text{O}_2 + 2\text{NO}_3^- + \frac{4}{3}\text{NO}_2^- + \frac{1}{2}\text{SO}_4^{2-} \right]_{4\text{m}}$$

Together these oxidants explain an additional 3% of observed methane oxidation, leaving 83% of CH_4 oxidation unaccounted for. As manganese and iron

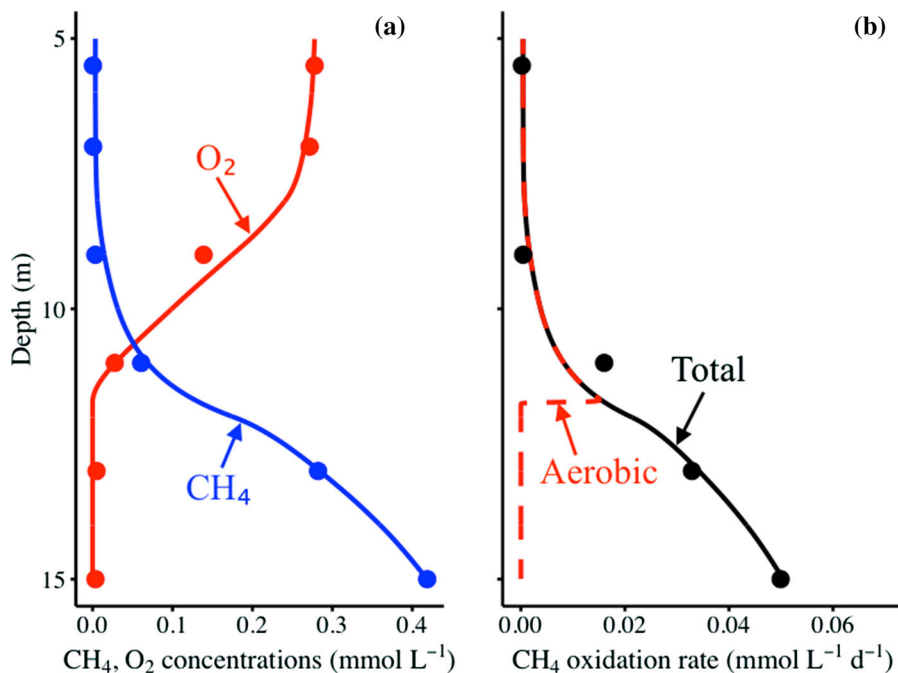


Fig. 2 Profiles of methane, oxygen, and methane oxidation rate in Lacamas Lake for 28 October, 2014. Circles represent observations, while lines represent model output. Panel **a** shows methane (blue) and oxygen (red) concentrations over a depth profile; panel **b** shows measured methane oxidation rate (black points), total modeled methane oxidation rates (aerobic plus

anaerobic; black line), and modeled aerobic methane oxidation rate (red). Oxygen measurements are accurate to ± 0.003 mmol L⁻¹ and methane measurements are accurate within 2.2% of actual values based on the coefficient of variation of repeat standards

Table 2 Concentrations of conventional electron acceptors measured at 4 m depth in Lacamas Lake (representing the boundary conditions for the reactive transport model)

Terminal electron acceptor	Concentration (mmol L ⁻¹)
Oxygen	0.277
Sulfate	0.090
Nitrate	0.020
Nitrite	0.002

oxides occur in the water column as particulates, they are subject to different transport processes (e.g., sinking) and cannot be incorporated within G, which only represents solutes. Nevertheless, estimates of reduced Mn and Fe accumulation in the water column, based on biweekly profiles, show that the contribution of these species to water column methane oxidation is likely to be negligible. Assuming all iron and manganese reduction is coupled to methane oxidation, the maximum rate at which Fe²⁺ and Mn²⁺ accumulate in the hypolimnion (approximately 0.2 kmol

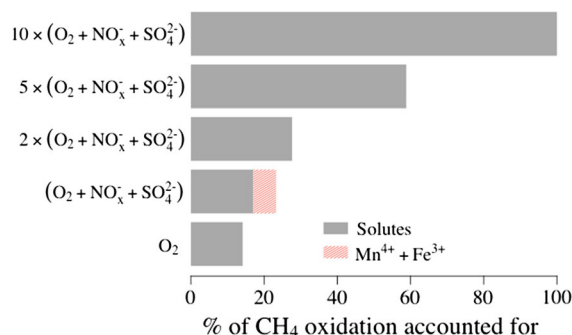


Fig. 3 Fraction of methane oxidation that can be explained when assuming different oxidant concentrations at the thermocline. The bottom gray bar represents a simulation considering observed oxygen concentrations only, while the gray bar immediately above also includes observed NO₂⁻, NO₃⁻, and SO₄²⁻ concentrations. The top three gray bars represent simulations with multiples of all the observed oxidants. In addition, the maximum fraction of oxidation that can be explained by manganese and iron reduction is depicted by a pink bar. Mn⁴⁺ and Fe³⁺ are not depicted in the scenarios where we multiply solute oxidant concentrations at the upper bound of the model because oxidized Mn and Fe exist in particulate form

Mn d^{-1} and 2.2 kmol Fe d^{-1}) can only explain 1.0 and 5.3% of the observed methane oxidation, respectively (Fig. 3). Our simulations suggest that about 10 times the solute oxidant concentration that is present at the thermocline is required to explain the methane consumption observed (Fig. 3). This raises the question: is there another important yet elusive electron acceptor mediating AOM?

Results from this reactive-transport modeling exercise as well as observations reported in previous studies both point to an unresolved pathway for AOM. While a balanced electron budget was constructed for eutrophic, monomictic Lake Rotsee based on estimations of turbulent diffusive transport, ultimately the authors concluded that “the question of which species are involved in methane oxidation could not be solved completely” (Schubert et al. 2010). In meromictic, oligotrophic Lake Gek-Gel, relatively high rates of anaerobic methane oxidation were observed within the sediment, but did not line up with the sulfate reducing zone leaving the dominant electron acceptor unidentified (Pimenov et al. 2010). In meromictic Lake Matano, the authors estimate that Fe and Mn must be recycled several times in the water column (i.e., across the oxycline) to balance the upward flux of CH_4 (Crowe et al. 2011), but whether this mixing is actually occurring is unknown. In eutrophic, meromictic Lake Lugano, bottle assays from the anoxic hypolimnion reveal greater rates of oxidation than can be explained by O_2 , SO_4^{2-} , NO_3^- , NO_2^- , and Fe^{3+} concentrations (Blees et al. 2014). While Lake Lugano’s methane oxidation budget is dominated by microaerophilic oxidation at the oxic-anoxic interface, the authors could not rule out AOM in deeper waters, asserting that “further investigation is required to ascertain potential anaerobic modes of CH_4 oxidation in Lake Lugano’s anoxic hypolimnion” (Blees et al. 2014).

Recent work has linked methane oxidation in anoxic lake waters to instantaneous O_2 production via photosynthetic algae (Milucka et al. 2015), but the methane oxidation incubations we report for Lacamas Lake were conducted in the dark. Additionally, the Secchi depths at Lacamas Lake are generally quite shallow (mean summertime depth of 1.4 m, Carlson et al. 1985) such that no photosynthetically active radiation can reach 12–15 m depth (where the highest rates of oxidation were observed). Redox cycling can also sometimes be “cryptic” whereby rapid co-occurring reactions can result in an apparent lack of

particular oxidants simply because the oxidant is turning over so quickly (Canfield et al. 2010). Nevertheless, cryptic oxidation reactions still require some source of oxidant (such as sulfide oxidation linked to nitrate/nitrite reduction as in the case of the cryptic N and S cycle in oceanic oxygen minimum zones, Canfield et al. 2010). In the electron budgeting exercises presented here, however, we could not identify a potential oxidant source using traditional electron acceptors.

Are organic acids elusive electron acceptors?

While it is well known that organic matter functional groups can accept electrons during fermentation-based molecule dismutation, a growing body of work reports the capacity for organic acids to serve as external TEAs in redox sensitive biogeochemical reactions (Lovley et al. 1996; Fimmen et al. 2007; Martinez et al. 2013). Experimental organic acid additions have been shown to limit freshwater CH_4 emissions in bogs (Blodau and Deppe 2012) and wetlands (Keller et al. 2009). Quinones, often considered a model redox-sensitive organic acid functional group, have recently been reported to serve as important electron acceptors in a variety of settings, such as peat soils (Lipson et al. 2010), freshwater sediments (Kappler et al. 2004), and periodically anoxic environments (Klöpffel et al. 2014). In addition to quinones, two independent studies found that nonquinone organic acid functional groups were responsible for anywhere between 44 and 58% of total electron transfer capacity (as cited in Martinez et al. 2013). Organic acids may thus limit aquatic CH_4 emissions to the atmosphere by extending the redox ladder, limiting the development and persistence of the highly reducing conditions required for methanogenesis (Cervantes et al. 2000).

In addition to extending the redox ladder, organic acids may also function as an intermediary by shuttling electrons across redox gradients in space or time to oxidize reduced species that are subsequently employed in AOM (Klöpffel et al. 2014). In the context of lakes and reservoirs, organic TEAs may be replenished on seasonal timescales wherein lake turnover drives the oxidation of organic acids both within the hypolimnion and at the sediment water interface, as has been observed in a northern bog undergoing seasonal oxic-anoxic cycles (Heitmann

et al. 2007). Furthermore, particle-associated organic TEAs may be supplied to anoxic waters in stratified lakes and reservoirs throughout the year with organic matter sinking across the oxycline. In this way, organic acids could also function to regenerate more common oxidants, such as iron oxyhydroxides and thiosulfate (via oxidation of Fe^{2+} and H_2S , Lovley et al. 1996; Heitmann and Blodau 2006; Saxton et al. 2016). Such indirect oxidation could give rise to complex reaction networks, for example by linking organic acid-rich environments (like sediments or sinking particles) with methane dissolved in the water column through intermediary species (e.g., iron).

Alternatively—or in addition—organic acids may serve as direct electron acceptors in the anaerobic oxidation of methane diffusing from freshwater sediments. Reductive dehalogenation of fluvic acids has recently been posited to fuel AOM in a permanently ice covered lake (Saxton et al. 2016) and AQDS, a model quinone organic acid, was recently shown to serve as an electron acceptor for ANME-mediated methane oxidation in marine sediments (Scheller et al. 2016). Recent work in wetland sediments also provides spectroscopic and incubation-based evidence for organic acid mediated AOM (in both the natural organic matter pool and in response to Pahokee Peat and AQDS amendments, Valenzuela et al. 2017). Quinone-fueled AOM is more thermodynamically-favorable than several common methanotrophic metabolisms that use inorganic electron acceptors (Table 1). This is particularly relevant for organic-rich (i.e., eutrophic) systems, as organic acids have been shown to be a major constituent of DOM in natural waters (>75% of all freshwater DOC on average, Perdue and Ritchie 2003), and quinoid functional groups are ubiquitous (Fimmen et al. 2007).

The dearth of known inorganic TEAs in the Lacamas water column late in the stratified season, in concert with an abundance of organic material (Deemer et al. 2011), lead us to hypothesize that organic acids act as important electron acceptors for AOM at this site. Although we are unable to test this hypothesis with currently available data, the observed DOC profile (Supplementary Fig. 5) indicates one or more DOC source(s) at depth (e.g., dissolution of sinking and resuspended particles, benthic DOC flux) and a sink(s) at the thermocline. We estimate that the standing DOC pool alone could support the methane oxidation we observed for somewhere between 0.3

and 2.75 days depending on the organic acid stoichiometry (AQDS vs. p-benzoquinone), and it is likely that this DOC is augmented substantially by other DOC sources, including diffusion from sediments, dissolution of sinking particles, dissolution of resuspended particles—Lacamas has a pronounced nepheloid layer—and cycling across the thermocline. At steady state, we estimate that $0.41\text{--}3.87 \text{ mol C m}^{-2} \text{ day}^{-1}$ would be needed to sustain the oxidation rates we observed. While this C flux is probably too large to sustain annually via primary production, we expect that the high methane oxidation rates we observed are transitory, occurring over a period of several weeks while the reservoir is turning over (as much lower rates of oxidation have been measured during other times of year, van Grinsven unpubl. data). Characterizing DOC and POC dynamics would be a key first step to undertaking a quantitative evaluation of the hypothesis that organic acids play an important role in modulating methane emissions from eutrophic lakes and reservoirs.

Future work: a call to researchers

Despite observations consistent with organic acid-mediated AOM in Lacamas Lake and several other systems, direct evidence of this pathway is lacking (but see Scheller et al. 2016; Valenzuela et al. 2017) and testing for this is an important direction for future work. We have identified several avenues of research that would help the field to advance towards a fuller understanding of AOM more generally and organic acid-mediated AOM in particular.

1. Continued effort should aim to better characterize the capacity for inhibitor and isotope-based tools to uncover AOM dynamics. Currently, specific methane oxidation inhibitors that target AOM pathways have yet to be identified. Future work should aim to identify inhibitors that act solely on AOM pathways so that the magnitude and controls on AOM can be better elucidated. This is not a trivial task given that anaerobic methanotrophic archaea can use a reversed methanogenesis pathway (Borrel et al. 2011) making it likely that many of the enzymes that inhibitors target are involved in both AOM and methanogenesis (as is the case with bromoethanesulfonate, Nauhaus

- et al. 2005). Isotope-wise, while methanotrophs generally preferentially oxidize lighter methane, evidence from SO_4^{2-} -mediated AOM in marine sediments suggests that AOM may either enrich or deplete the $^{13}\text{CH}_4$ pool depending on the availability of SO_4^{2-} (Yoshinaga et al. 2014)—a pattern that is consistent with observations in an ice covered lake (Saxton et al. 2016). Isotope labeling experiments across a range of electron acceptor availabilities in systems where AOM pathways are known to dominate would provide a useful reference for researchers aiming to use spatial or temporal patterns in isotopic signatures to infer the presence of AOM.
2. Organic acids have been shown to be ecologically relevant in freshwaters (Lennon et al. 2013). Precise characterization of DOM in natural waters (e.g. Kellerman et al. 2015) and continued efforts to identify redox couples (e.g. Fimmen et al. 2007) would help to quantify substrate availability for microbes that employ organic acids as electron acceptors. Such information would give researchers a better idea of how to amend bottle assays to target organic acid-mediated AOM (rather than relying on stock DOM that may or may not be representative of natural DOM). Also, given the tight coupling between DOM and POM, it is also important to measure the distribution and fluxes of these organic matter phases (particularly resuspension fluxes and fluxes across the sediment water interface). This would help when developing and constraining numerical models, which provide invaluable insights into biogeochemical dynamics.
 3. The relative role of organic acids as direct electron acceptors (Scheller et al. 2016) versus electron shuttles (Heitmann and Blodau 2006; Martinez et al. 2013; Klüpfel et al. 2014) in supporting AOM should be examined in freshwater ecosystems. This question could be addressed using bottle assays (and appropriate methanogenesis inhibitors if necessary) to incubate water with known AOM with amendments of both organic acids and reduced intermediaries such as H_2S , Mn^{2+} and Fe^{2+} . If organic acids only facilitate AOM in the presence of intermediary amendments, then it may be that their role as electron shuttles is more important than their role as direct electron acceptors.
 4. A diverse array of bacteria and archaea are known to be capable of reducing organic acids (Martinez et al. 2013). Scheller and colleagues recently showed that ANME-2 archaea groups were involved in direct organic acid (AQDS)-mediated AOM and that the process was decoupled from a sulfate reducing partner (Scheller et al. 2016). Still, the potential for other archaea and bacteria to couple organic acid reduction to methane oxidation is currently unknown. It is likely that groups other than ANME can couple organic acid reduction to AOM given that ANME were barely detected in wetland sediments undergoing organic acid mediated AOM (Valenzuela et al. 2017). Characterizing the microbial communities responsible for AOM linked to organic acids, as well as associated biochemical parameters (e.g., half-saturation coefficients, rate constants), is key to understanding these pathways. State-of-the-art molecular tools (e.g., metagenomics, metatranscriptomics) together with classical incubation and culturing approaches will prove invaluable to this end.

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