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Anaerobic oxidation of methane by aerobic methanotrophs in sub-Arctic lake sediments

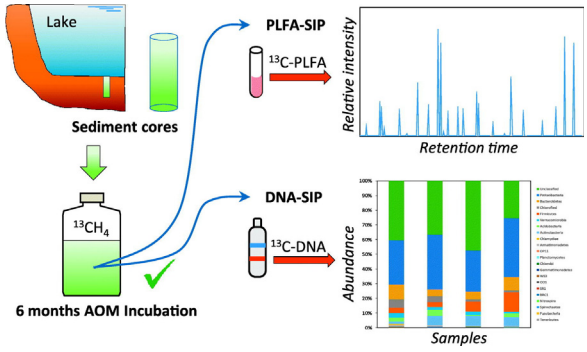
Karla Martinez-Cruz [a,b], Mary-Cathrine Leewis [c], Ian Charold Herriott [c], Armando Sepulveda-Jauregui [a], Katey Walter Anthony [a], Frederic Thalasso [a,b], Mary Beth Leigh [c,*]

[a] Water and Environmental Research Center, Institute of Northern Engineering, University of Alaska Fairbanks, 306 Tanana Loop, 99775 Fairbanks, AK, USA
[b] Biotechnology and Bioengineering Department, Cinvestav, 2508 IPN Av, 07360, Mexico City, Mexico
[c] Institute of Arctic Biology, University of Alaska Fairbanks, 930 N Koyukuk Dr, 99775Fairbanks, AK, USA

HIGHLIGHTS

- Anaerobic oxidation of methane (AOM) in a sub-Arctic lake sediment was explored.
- Incubations over 6 months with $^{13}CH_4$ confirmed AOM and ^{13}C-DNA enrichment.
- DNA- and PLFA-SIP suggest the genus *Methylobacter* was involved in AOM.
- *Methylophilus*, *Ralstonia*, *Syntrophus* and *Albidiferax* were also potentially involved.
- C incorporated by AOM flowed out broadly through the microbial community.

GRAPHICAL ABSTRACT



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ABSTRACT

Anaerobic oxidation of methane (AOM) is a biological process that plays an important role in reducing the CH_4 emissions from a wide range of ecosystems. Arctic and sub-Arctic lakes are recognized as significant contributors to global methane (CH_4) emission, since CH_4 production is increasing as permafrost thaws and provides fuels for methanogenesis. Methanotrophy, including AOM, is critical to reducing CH_4 emissions. The identity, activity, and metabolic processes of anaerobic methane oxidizers are poorly understood, yet this information is critical to understanding CH_4 cycling and ultimately to predicting future CH_4 emissions. This study sought to identify the microorganisms involved in AOM in sub-Arctic lake sediments using DNA- and phospholipid-fatty acid (PLFA)-based stable isotope probing. Results indicated that aerobic methanotrophs belonging to the genus *Methylobacter* assimilate carbon from CH_4, either directly or indirectly. Other organisms that were found, in minor proportions, to assimilate CH_4-derived carbon were methylotrophs and iron reducers, which might indicate the flow of CH_4-derived carbon from anaerobic methanotrophs into the broader microbial community. While various other taxa have been reported in the literature to anaerobically oxidize methane in various environments (e.g. ANME-type archaea and *Methylomirabilis Oxyfera*), this report directly suggest that *Methylobacter* can perform this function, expanding our understanding of CH_4 oxidation in anaerobic lake sediments.

* Corresponding author.
E-mail addresses: karla.martinez@umag.cl (K. Martinez-Cruz), mcleewis@alaska.edu (M.-C. Leewis), icherriott@alaska.edu (I.C. Herriott), asepulvedajauregui@alaska.edu (A. Sepulveda-Jauregui), kmwalteranthony@alaska.edu (K.W. Anthony), thalasso@cinvestav.mx (F. Thalasso), mbleigh@alaska.edu (M.B. Leigh).

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1. Introduction

Lakes cover a small proportion of Earth's surface (ca. 3.6%, Verpoorter et al. 2014; Downing et al., 2006), but are recognized to play an important role in the global carbon cycle by contributing up to 16% of the total methane (CH_4) emissions to the atmosphere (Bastviken et al., 2011; Holgerson and Raymond, 2016; Saunois et al., 2016) compared to less than 1% by oceans (Rhee et al., 2009). By surface area, almost half of lakes are located in Artic and sub-Arctic regions and, among them, small ponds and thermokarst lakes are known to emit large quantities of CH_4 (Wik et al., 2016). Thus, obtaining a clear understanding of the CH_4 cycling in small Artic and sub-Arctic lakes would help to accurately determine their contribution to the global carbon budget.

Methane is produced biologically from simple carbon molecules under anoxic environments such as lake sediments (Heslop et al., 2015; Winfrey and Zeikus, 1979). When oxygen is available, the CH_4 flux from sediment is reduced, mainly at the oxycline, by aerobic methanotrophy, which is known to significantly mitigate the CH_4 release to the atmosphere (Bastviken et al., 2002; Lofton et al., 2014; Martinez-Cruz et al., 2015). Under anoxic conditions, anaerobic oxidation of methane (AOM) can occur in the presence of alternative electron acceptors such as sulfates, nitrates, iron, manganese, and humic substances, among others (Caldwell et al., 2008; Smemo and Yavitt, 2011; Scheller et al. 2016; Thauer and Shima, 2008). AOM has been observed in anaerobic sediments and soils (Blazewicz et al., 2012; Boetius et al., 2000; Deutzmann et al., 2014; Reeburgh, 1976; Schubert et al., 2011; Zehnder and Brock, 1980) and is also known to significantly constrain CH_4 emissions to the atmosphere (Reeburgh, 2007; Segarra et al., 2015; Sivan et al., 2011; Smemo and Yavitt, 2011). In ocean sediments, AOM has been observed coupled to sulfate-reduction (AOM-SR), and some characteristics of microbes performing AOM-SR have already been described (Hallam et al., 2004; Thauer, 2011; Wegener et al., 2016). However, the mechanism of AOM and the identity of the organisms performing it in lakes remains unclear. Considering the large CH_4 emissions from Artic and sub-Arctic lakes, it is important to characterize the processes and microorganisms controlling CH_4 oxidation within these lakes in order to better understand and predict future CH_4 emissions to the atmosphere.

Among the microorganisms known to perform AOM, the methanogen-like anaerobic methanotrophic (ANME) archaea have been found to perform AOM in syntrophic association with sulfate-reducing bacteria (Boetius et al., 2000; Hoehler et al., 1994; Valentine, 2002) and to efficiently remove CH_4 from anoxic oceanic sediments and water (Knittel and Boetius, 2009). However, with few exceptions, such as areas prone to acid rain or in watersheds with acid sulfate soils and/or rocks, sulfate (SO_4^{-2}) concentrations in most lakes are typically too low for sulfate-reducing processes to be thermodynamically favorable (Schubert et al., 2011; Smemo and Yavitt, 2007). Anaerobic iron (Fe^{+3}) and manganese (Mn^{+4}) reduction coupled to AOM are more favorable reactions and have been demonstrated to occur and to be a significant process in lakes (Nordi et al., 2013; Sivan et al., 2011). Yet little is known about the microorganisms involved in AOM coupled to Fe^{+3} and Mn^{+4}; one study revealed that the most abundant microorganisms in the sediments after incubations of AOM linked to Fe^{+3} and Mn^{+4} were affiliated with the archaeal marine benthic group D and ANME (Beal et al., 2009).

AOM coupled to denitrification (AOM-D) is thermodynamically more favorable than AOM-SR. It was first demonstrated in enrichment cultures (Islas-Lima et al., 2004; Raghoebarsing et al., 2006) and has since been reported in lake sediments with a high input of NO_3^- (Deutzmann and Schink, 2011). *Methylomirabilis oxyfera*, a member of NC10 phylum, and *Candidatus Methanoperedens nitroreducens*, an ANME-type archaeon, have been identified as microorganisms capable of performing AOM-D (Ettwig et al., 2010; Haroon et al., 2013). Although NO_3^- and NO_2^- are lacking in most anoxic sediments (Smemo and Yavitt, 2007), populations of NC10 bacteria have been detected, in low abundance, in both semi-oxic and anoxic sediment layers of lakes (Beck et al., 2013; Deutzmann et al., 2014). Likewise, some studies in lakes have shown the presence of aerobic methane-oxidizing bacteria (MOB) in anoxic zones (Deutzmann et al., 2014), mainly those methanotrophs belonging to Gammaproteobacterial MOB (Biderre-Petit et al., 2011; Blees et al., 2014; Kalyuzhnaya et al., 2013; Oswald et al., 2016). Furthermore, some Gammaproteobacterial MOB from lake sediments were recently found to be active under anoxic conditions (Oswald et al., 2016), challenging the long-term dogma of the 'strictly' aerobic nature of these organisms (Chistoserdova, 2015; Kalyuzhnaya et al., 2013).

To contribute to the understanding of the CH_4 cycle, we sought to identify microorganisms involved in AOM in sediments of a sub-Arctic lake known to emit large amounts of CH_4. To achieve this, we used DNA- and phospholipid fatty acid (PLFA)-based stable isotope probing (SIP), quantitative (Q)-PCR, and amplicon-based sequencing to track carbon derived from $^{13}CH_4$ through the active anaerobic microbial community from the sub-Arctic lake sediments incubated without the addition of external electron acceptors. Results indicate that aerobic methanotrophs belonging to the genus *Methylobacter* might be responsible for anaerobically oxidizing about 32% of the CH_4 being produced at surficial sediments, further constraining the CH_4 emitted to the atmosphere. However, further investigation is needed to elucidate which processes may provide O_2 required for the aerobic CH_4 oxidation carried out mainly by *Methylobacter*.

2. Material and methods

2.1. Sample collection and physical and chemical analysis

Lake Vault is a thermokarst lake located near Fairbanks, Alaska, formed in yedoma permafrost with active thaw-bulb, emitting about 41 g CH_4 m^{-2} y^{-1} (Sepulveda-Jauregui et al., 2015). The main morphological and limnological characteristics were described by Heslop et al. (2015) and Sepulveda-Jauregui et al. (2015).

In March 2013, three sediment cores were collected from the center of Lake Vault; 65.0292°N, 147.6984°W. The cores with overlying water were collected in polycarbonate tubes (7.5 cm diameter) using a piston hammer corer (Aquatic Research Instruments, Hope, ID, USA) and were sealed without headspace. Cores were immediately transported to the laboratory and stored in the dark at 4 °C until processing and analysis, within 24 h after collection.

Sediment cores were split in half vertically, one half of each core was immediately sealed with four layers of O_2-and-moisture-barrier film (Krehalon PC101, Filcon, Clare, MI, USA) to impede gas exchange and was stored at 4 °C until initiation of incubations tests. The other half was subsampled for physicochemical analysis. Martinez-Cruz et al. (Unpublished results) demonstrated higher AOM rates in the first centimeters of sediment cores from sub-Arctic and temperate lakes. Thus, this study is focused on the top 2.5 cm of Lake Vault sediment cores.

Dissolved nitrate (NO_3^-) and nitrite (NO_2^-) in pore water were determined based on a colorimetric technique according to standard methods (APHA, 1999) with a detection limit of 1 μM. Sulfate (SO_4^{2-}) concentration was measured with ion chromatograph (150/4.0 mm ID column, eluent 3.2 mM Na_2CO_3, 1 mM $NaHCO_3$, detection limit 5 μM; electrical conductivity detector; Dionex, Sunnyvale, CA, USA). Total iron (Fe) and total manganese (Mn) were determined according to standard methods (APHA, 1999).

2.2. Stable isotope probing incubations

Stable isotope probing (SIP) incubations were conducted by pooling and homogenizing 200 ml of sediments from the top 2.5 cm section of the cores and transferring them into 130 ml of anaerobic water while flushing with ultrahigh purity N_2 (99.999%, UHP N_2, AirGas, Radnor,