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Discussions

Spatial variability of diploptene δ^{13}C values in thermokarst lakes: the potential to analyse the complexity of lacustrine methane cycling

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12157

Abstract

Cryospheric changes in northern high latitudes are linked to significant greenhouse gas flux to the atmosphere, including methane release that originates from organic matter decomposition in thermokarst lakes. The connections between methane production in sediments, transport pathways and oxidation are not well understood and this has implications for any attempts to reconstruct methane production from sedimentary archives. We assessed methane oxidation as represented by methane oxidising bacteria across the surface sediments of two interior Alaska thermokarst lakes in relation to methane emissions via ebullition (bubbling). The bacterial biomarker diploptene was present and had low $\delta^{13}C$ values (lower than $-38‰$) in all sediments analysed, suggesting methane oxidation was widespread. The most $\delta^{13}C$-depleted diploptene was found in the area of highest methane ebullition emissions in Ace Lake ($\delta^{13}C$ diplotene values between -68.2 and $-50.1‰$), suggesting a positive link between methane production, oxidation, and emission in this area. In contrast, significantly less depleted diploptene $\delta^{13}C$ values (between -42.9 and $-38.8‰$) were found in the area of highest methane ebullition emissions in Smith Lake. Lower $\delta^{13}C$ values of diploptene were found in the central area of Smith Lake (between -56.8 and $-46.9‰$), where methane ebullition rates are low but methane diffusion appears high. Using $\delta^{13}C$-diplotene as a proxy for methane oxidation activity, we suggest the observed differences in methane oxidation levels among sites within the two lakes could be linked to differences in source area of methane production (e.g. age and type of organic carbon) and bathymetry as it relates to varying oxycline depths and changing pressure gradients. As a result, methane oxidation is highly lake-dependent. The diploptene $\delta^{13}C$ values also highlight strong within-lake variability, implying that single-value, down-core records of hopanoid isotopic signatures are not secure indicators of changing methane flux at the whole-lake scale.

1 Introduction

Thermokarst and thermokarst-affected lakes (those formed and/or influenced by thaw and collapse of ice-rich ground) are now recognized as important past and present sources of methane flux to the atmosphere (Shirokova et al., 2012; Walter et al., 2006, 2008; Wik et al., 2013). Under current scenarios of projected future climate warming in regions sensitive to thaw (Colins et al., 2013), these lakes are expected to remain a source of methane emissions to the atmosphere (Vincent et al., 2013). Predictions of the future contribution they will make to the dynamic global carbon cycle and any estimations of past emission rates are largely based on measurements recorded over the last 15 years (e.g. Brosius et al., 2012; Walter Anthony et al., 2014). Long-term (i.e. Holocene) variations in lake-derived methane flux to the atmosphere and changes in emissions during discrete climatic events in the past are less well understood (but see Walter Anthony et al., 2014; Walter et al., 2007b). A proxy for past gas flux from lakes would be an important development in better understanding long term carbon cycling, but we are far from understanding within-lake methane dynamics well enough for such a proxy to yet be reliable.

The broad term "thermokarst lakes" encompasses a complex range of lakes types associated with different geographical and geomorphological settings in permafrost regions. Methane production within these lakes and fluxes to the atmosphere vary with lake type. Walter et al. (2008) and Brosius et al. (2012) divide thermokarst lakes into two main categories: yedoma lakes and non-yedoma lakes, where yedoma refers to late Pleistocene deposits of organic- and ice-rich silt, typically several or more metres deep (Zimov et al., 2006; Schirrmeister et al., 2013).

Methane production in thermokarst lakes can be classified by production type: production that occurs in anoxic surface sediments, as is common in most freshwater lakes and reservoirs, and production that occurs in deeper sediments, especially along the boundary of the "thaw bulb", which is specific to thermokarst lakes (Fig. 1). Anoxia is caused by oxygen depletion associated with microbial decomposition of organic mat-

ter. Anoxic conditions are enhanced by thermal stratification in the water column and/or by rapid sedimentation that buries labile organic material before it can be processed at the sediment surface. A common trait of thermokarst lakes is methane production via mineralisation of organic carbon from sources not found in other lakes. For example, methane emissions can occur where thermokarst-induced erosion leads to large-scale slumping of banks into the littoral zone; material is typically of Holocene age, but may be older (Fig. 1). As well as the production from slumped material, yedoma lakes may feature high methane emissions related to the microbial processing of older, labile carbon in the deep thaw bulb (talik, i.e., an area of thawed permafrost sediment underneath the lake). Walter Anthony and Anthony (2013) suggest that yedoma thermokarst lakes typically produce more methane than non-yedoma thermokarst lakes owing to a higher availability of labile carbon in thick, thawed yedoma sequences.

Once produced, methane can be transported to the atmosphere through a number of pathways: ebullition (bubbling), turbulent diffusion and plant mediated transport (Bastviken, 2004). Several studies have focused on these emission pathways, assessing methane production and emission levels in freshwater environments (e.g. Bastviken, 2004; Bastviken et al., 2011; Delsontro et al., 2011; Joyce and Jewell, 2003).

Thermokarst-specific methane ebullition seeps have been observed and measured using GPS mapping and submerged bubble traps and described as persistent, spatially explicit fluxes at the water–air interface (Sepulveda-Jauregui et al., 2015; Walter et al., 2006, 2008; Walter Anthony and Anthony, 2013). Ebullition seeps are thought to be fairly stable due to the development of conduits or "bubble tubes" (Greinert et al., 2010; Scandella et al., 2011), which are point sources from which methane is emitted to the atmosphere repeatedly at the sediment–water interface. Nearly always, such seeps are densest near to actively eroding lake margins, which we call the "thermokarst zone". Here, methanogenesis is high due to thermokarst-specific sources of methane production: thawing of fresh talik and bank collapse (Fig. 1; Kessler et al., 2012). Walter Anthony et al. (2010) postulate that most methane production that is specific to thermokarst lakes is transported to the atmosphere via seep ebullition (due to high

rates of methane production in dense, thick talik sediments), although the diffusive flux component can be relatively high in older, more stable thermokarst lakes that have accumulated Holocene-aged organic carbon in near-surface sediments.

Less work has focused on methane production in surficial sediments of thermokarst lakes, dissolution and diffusion of methane from the sediments to the water column, and resultant diffusive emission, particularly in thermokarst zones. This paper reports an analysis of carbon isotopes in sedimentary bacterial biomarkers in relation to different forms of atmospheric methane flux from two lakes near Fairbanks, Alaska, with the aim of improving our understanding of methane cycling in thermokarst lake systems and assessing the effectiveness of biomarkers as a proxy for methane cycling in lakes.

1.1 The link between methane ebullition and methane diffusion from sediments

A significant fraction of methane produced in lake sediments can be oxidized and recycled within the lake, processes that offset methane emissions. Methane that has diffused from the sediments is subject to aerobic microbial oxidation by bacteria (Bastviken et al., 2002; Liebner and Wagner, 2007; Trotsenko and Khmelenina, 2005). Aerobic methane oxidation (MO) is thought to considerably reduce methane emissions from water bodies (Reeburgh, 2007). MO studies in lakes have mostly been carried out under stratified water column conditions (Bastviken et al., 2002; Kankaala et al., 2006). As with diffusive methane flux (Sepulveda-Jauregui et al., 2015), little work has focused on aerobic MO in thermokarst lakes (Martinez-Cruz et al., 2015). Understanding the link between MO and observed fluxes is crucial for developing a proxy for past methane production in thermokarst lakes.

In studies based on deep marine environments there is a correlation between widespread methane, released via cold seeps through sediments, and MO, as indicated by the presence and $\delta^{13}C$ values of specific bacteria and compounds (Elvert et al., 2001a; Pancost et al., 2001, 2000b). In these environments both anaerobic (Alperin and Hoehler, 2010; Briggs et al., 2011) and aerobic (Birgel and Peckmann,

2008; Elvert and Niemann, 2008) MO processes have been identified and are important for mediating methane flux to the atmosphere. As well as a link between methane ebullition seeps and methane diffusion in deep marine settings, a study carried out in a shallow (9 m) near-shore bight linked the formation of bubble tubes with increased methane diffusing from the sediments (Martens and Klump, 1980), the argument being that bubble tubes create an increased surface area that enhances methane diffusion, even though methane transported via ebullition is taken directly to the atmosphere and is not subject to oxidation. While derived from different environments than thermokarst lakes, the deep and shallow marine results suggest a positive relationship between transport via ebullition and methane diffusion from sediments, which may also occur in thermokarst lakes.

He et al. (2012) provide evidence that suggests a possible correlation between a coal-bed sourced methane ebullition seep and MO in the non-yedoma thermokarst lake, L. Qalluuraq, Alaska. The highest MO potentials occurred near the coal-bed sourced ebullition seep and were associated with the presence of type I MOB in the sediments at the seep location. He et al. (2012) also observed high spatial variability of MO potentials and methanotroph communities and highlighted the need for further investigation of MO in thermokarst lakes.

In contrast, based on δ^{13}C and δD stable isotope values and radiocarbon ages of methane in bubbles, Walter et al. (2008) and Walter Anthony et al. (2014) suggest that methane emitted by ebullition originating in deep thaw-bulb sediments by-passes aerobic MO and that the majority of deep-sourced methane is transported through ebullition seeps as opposed to escaping sediments via diffusion. We therefore have two contrasting conceptual models (hypotheses): an enhancement model and a by-pass model. In the enhancement model, the thermokarst zone of a lake, where ebullition seeps are most abundant, would have higher levels of deep-sourced methane diffusion from sediments when compared with "quiescent" areas that are virtually ebullition seep free. In the by-pass model, where diffusion of deep-sourced methane out of sediments is thought to be minimal, we expect no difference between thermokarst-zone and lake-

12163

centre diffusion of deep-sourced methane from sediments, or, conceivably, less diffusion in the seep-rich area. A potential confounding factor is diffusion of methane that is formed in near-surface sediments, which can have variable and contrasting patterns across lakes, independent of spatial patterns of ebullition seeps.

Past methane emissions may be addressed qualitatively by using indirect proxies, for example, features related to the cycle of methane through the lacustrine food web. Biogenic methane has highly depleted δ^{13}C values (usually −50 to −80‰, Whiticar, 1999), depending on the methane production pathway and substrate availability. These depleted δ^{13}C values can be traced through the food web, for example, in low-level heterotrophs such as invertebrates. Previous studies have linked depletion in the δ^{13}C values at various stages in the food web to the incorporation of carbon from of methane oxidising bacteria (MOB, van Hardenbroek et al., 2010; Jones and Grey, 2011; Sanseverino et al., 2012). Recent studies have demonstrated that some chironomid (nonbiting midge) taxa utilise MOB as a food source within lakes (Deines et al., 2007; van Hardenbroek et al., 2010). In thermokarst lakes, depleted δ^{13}C values in larvae and fossil head capsules have been linked to increased methane flux (van Hardenbroek et al., 2013). Wooller et al. (2012) also interpret negative shifts in δ^{13}C values of fossil chironomids and daphnia as an increase in methane availability.

MOB have been identified in sediments from a wide range of terrestrial and aquatic environments. They are known to synthesise a number of specific compounds that can be isolated. In particular, the compound diploptene (17 β(H), 21 β(H)-hop-22 (29)-ene), a hopanoid hydrocarbon derived from a range of bacterial sources, has been identified as a methanotrophic biomarker via low δ^{13}C values in marine sediments and microbial mats associated with methane seeps (Elvert et al., 2001b; Pancost et al., 2000a, b) as well as Holocene peat (van Winden et al., 2010; Zheng et al., 2014). Diploptene and the related diplopterol have been used to establish past patterns of MO from marine sediment records (Jahnke et al., 1999; Pancost et al., 2000a) as well as lake sediments (Spooner et al., 1994; Schouten et al., 2001), and peat deposits (Kip et al., 2010; van Winden et al., 2012; Zheng et al., 2014).

12164

To oxidise methane effectively, MOB require access to dissolved methane in sediments and lake water. The assumption is, therefore, that isotopic depletion at or near the base of the food web indicates oxidation of dissolved methane. The extent to which isotopic signals can be used as a proxy for past methane ebullition flux in thermokarst lakes depends on the relationship between ebullition and diffusion and the sensitivity of the isotope signal to changing methane supply. In order to investigate these issues, we applied the approach used to identify MO at deep marine vents and seeps – lipid biomarkers from bacteria – to different areas associated with known ebullition emission patterns in two Alaskan lakes. MOB are a more direct proxy for methane than organisms higher in the food chain, and their use should allow a better understanding of methane diffusion from sediments, particularly in areas of ebullition seeps. The presence and δ^{13}C values of diploptene were used firstly to establish if MO was occurring at levels detectable by biomarkers, and secondly to assess the degree of MO observed in areas characterized by different modes of methane production and transport to the atmosphere.

2 Regional context and study sites

Yedoma-like deposits that are similar to those described in, and common to, Siberia (Schirrmiester et al., 2011) can be found in Interior Alaska. These sediments can have a relatively high organic content (Péwé, 1975) and are rich in excess ice. Thermokarst lakes that develop in landscapes dominated by these deposits have been placed into the yedoma or non-yedoma types (as described above) in previous studies (Walter et al., 2008; Brosius et al., 2012; Sepulveda-Jauregui et al., 2015). Two lakes were sampled in April 2011 and July 2012 (Fig. 2). Ace L. represents a yedoma-type lake (Sepulveda-Jauregui et al., 2015), where the permafrost soils surrounding the lake and eroding into the lake along its NE margin are predominantly yedoma. Smith L. is classified as a non-yedoma lake in which Holocene-aged deposits are likely the main source of organic matter fuelling methane production.

Smith L. (64°51′55.92″ N, 147°52′0.70″ W; Fig. 2) is a shallow (≤ 4 m), productive lake located in Interior Alaska. It has a gentle bathymetric profile with average water depths between 1–3 m. The lake is not subject to a strong fetch or high energy inflow or outflow. Observations during the ice-free periods suggest high primary productivity, with blue/green algal blooms predominant throughout the summer months (K. L. Davies, personal observation, 2012). The lake likely originated by thermokarst processes (Alexander and Barsdate, 1971); comparisons of lake shorelines between the 1950 s and today suggest that segments of the southern and western margins have been actively thermokarsting during recent decades, and tilting trees currently lining the margin of a bay on the southeast shore are further evidence of localized thermokarst. Smith Lake is a useful study site as its shallow profile reduces the potential of production or storage of methane due to stratification. Ace L. (64°51′45.49″ N, 147°56′05.69″ W) is part of the Ace-Deuce Lake system (Alexander and Barsdate, 1974) situated within an area covered by the Pleistocene Gold Hill and Goldstream loess formations (Pewe, 1975). Ace L. is thermokarst in origin and formed through the thawing of ice bodies in the loess. The Ace-Deuce Lake system has high nutrient levels, and therefore Ace Lake can be described as a eutrophic lake with a strong seasonal nutrient cycle (Alexander and Barsdate, 1974).

3 Methods

3.1 Establishing sample regions

Walter Anthony and Anthony (2013) defined the "thermokarst" zone for a number of lakes, and we continue to use this definition here. The thermokarst zone was the region of active thermokarst margin expansion observed using historical aerial photographs obtained during the past 60 years. In most lakes, the density of ebullition seeps is higher in thermokarst zones compared to non-thermokarst zones (Walter Anthony and Anthony, 2013). In Ace and Smith L., ebullition emissions were quantitatively monitored

through a combination of winter-time ice-bubble surveys and bubble-trap flux measurements via previous studies (Sepulveda-Jauregui et al., 2015) and our own summertime bubble counts (Fig. 2). We obtained surface sediment cores well within the zone boundaries and as close to observed ebullition seep locations as possible. At Ace L., bubble counts may have been underrepresented due to fetch-mediated surface turbulence disturbing visual counts of bubbles. However this was an issue at all count sites, such that, any error encountered will be associated with the overall scale of emissions measured and not with bias between zones.

3.2 Methane monitoring

Ebullition gas samples were collected from the thermokarst zone in the manner described in Walter Anthony et al. (2012) for determination of bubble methane concentration, stable isotope analyses, and radiocarbon dating. Gases were collected from submerged bubble traps into 60 mL glass serum vials following Walter et al. (2008), sealed with butyl rubber stoppers, and stored under refrigeration in the dark until analysis in the laboratory. We measured methane concentration using a Shimadzu 2014 equipped with an FID at the Water and Environmental Research Centre at University of Alaska Fairbanks (UAF). We determined $\delta^{13}C_{CH_4}$, using a Finnegan Mat Delta V, and δD_{CH_4} on a Delta XP at Florida State University. Subsamples of gas were combusted to CO_2, purified, and catalytically reduced to graphite (Stuiver and Polach, 1977), and the $^{14}C/^{12}C$ isotopic ratios were measured by accelerator mass spectrometry at the Woods Hole Oceanographic Institution's National Ocean Sciences AMS Facility. Stable isotope compositions are expressed in δ (‰) = 103 $((R_{sample}/R_{standard})-1)$, where R is $^{13}C/^{12}C$ or D/H and standards refer to the Vienna Pee Dee Belemnite (VPDB) and Vienna Standard Mean Ocean Water (VSMOW), respectively. The analytical errors of the stable isotopic analyses are ±0.1‰ $\delta^{13}C$ and ±1.0‰ δD. We express radiocarbon data as percent modern carbon pmC(%) = $((^{14}C/^{12}C)_{sample}/(^{14}C/^{12}C)_{standard}) \times 100$,

12167

which is the percentage of $^{14}C/^{12}C$ ratio normalized to $\delta^{13}C = -25$‰ and decay corrected relative to that of an oxalic standard in 1950 (Stuiver and Polach, 1977).

3.3 Biomarker analysis

Surface sediment samples were retrieved using a gravity corer and the 0–5 cm sequence was extruded at 1 cm resolution and retained for analysis; the 1–2 cm slice was subsampled for biomarker analysis. Two sequential extractions were performed upon the samples. The first step was a modified Bligh and Dyer extraction (Bligh and Dyer, 1959). Briefly, buffered water was prepared adjusting a solution of 0.05 M KH_2PO_4 in water to pH 7.2 through the addition of NaOH pellets. Subsequently, a monophasic solvent mixture was made up with buffered water, $CHCl_3$ and MeOH (4 : 5 : 10 v/v). Samples were sonicated in Bligh-Dyer solvent mixture for 15 min and then centrifuged at 3000 rpm for 5 min. Supernatant was collected in a round bottom flask. This step was repeated twice and all supernatants were combined and dried to obtain the total lipid extraction (TLE) labelled TLE1. Post-extraction sediment residues were air-dried. The Bligh and Dyer post-extraction residues were sonicated in DCM for 15 min and then centrifuged at 3000 rpm for 5 min. This step was repeated first with DCM : MeOH (1 : 1, v/v) and then with MeOH. Supernatants were combined after every step of sonication-centrifugation to obtain TLE2. Both TLE1 and TLE2 were then combined to yield the final TLE.

The TLE was split into three fractions of increasing polarity using silica flash column chromatography (Oba et al., 2006; Pitcher et al., 2009). Silica gel columns (0.5 g, 60 Å particle size) were prepared and conditioned with 4 mL of n-hexane : ethyl acetate (3 : 1, v/v). Fractions were eluted with 3 mL of n-hexane : ethyl acetate (3 : 1, v/v) to obtain the simple lipid fraction, 3 mL of ethyl acetate to obtain glycolipids and 10 mL of MeOH to obtain phospholipids. The simple lipid fraction was further split into neutral lipid and the fatty acid fractions. The organic phase was then collected into a round bottom flask and Na_2SO_4 anhydrous was added until complete removal of water. Sil-

12168

ica gel columns (again, 0.5 g, 60 Å particle size) were prepared and conditioned with 4 mL of the recently prepared CHCl$_3$ sat solution. The simple lipid fraction was then loaded onto the column and subsequently, the neutral lipid fraction was eluted with 9 mL of CHCl$_3$ sat. Finally, the neutral lipids were separated into apolar and polar lipid fractions. Columns were prepared with approximately 0.5 g of activated alumina (Al$_2$O$_3$) and compounds eluted with 4 mL of *n*-hexane : DCM (9 : 1, *v*/*v*) and 3 mL of DCM : MeOH (1 : 2, *v*/*v*) to yield the two fractions, respectively. Here, we focus on analyses of the neutral lipid apolar fraction.

3.4 Compound identification and compound-specific δ^{13}C isotope analysis

GC-MS analyses were performed using a Thermoquest Finnigan Trace GC and MS. The GC was fitted with an on-column injector and the stationary phase was CP Sil5-CB. Detection was achieved with electron ionization (source at 70 eV, scanning range 50–580 Daltons). The temperature program consisted of three stages: 70–130 °C at 20 °C min^{-1} rate; 130–300 °C at 4 °C min^{-1}; and 300 °C, temperature held for 10 min.

Gas chromatography combustion isotope ratio mass spectrometry (GC-IRMS) was performed using a ThermoScientific Trace GC Ultra coupled to a Conflo IV interface and DeltaV mass Spectrometer. The GC conditions and program were the same as for GC-MS analyses. Calibration was achieved using CO$_2$ reference gas of known isotopic composition and sample δ^{13}C values were expressed against the standard VPDB. All measurements were performed in duplicate.

3.5 Mass balance equation

A carbon isotopic mass balance equation (Eq. 1), or two-part mixing model, was developed to evaluate the contribution of MOB to the total bacterial biomass, and therefore, the relative amount of oxidation occurring at each sample location. The resulting end

member values are given in Table 1. The equation is as follows:

$$f_{\text{mob}} = \frac{\delta^{13}C_{\text{dip}}\delta^{13}C_{\text{hetero\_hopane}}}{\delta^{13}C_{\text{mob\_hopane}} - \delta^{13}C_{\text{hetero\_hopane}}} \tag{1}$$

f_{mob} is the fraction of diploptene generated by MOB and $\delta^{13}C_{\text{dip}}$ is the stable carbon isotopic composition of diploptene in a given sample. $\delta^{13}C_{\text{hetero\_hopane}}$ is the δ^{13}C value of the hopanoids derived from heterotrophic bacteria, the inferred other primary source of hopanoids in this setting, and is expressed as the $\delta^{13}C_{\text{bacterial}}$ biomass − $\Delta^{13}C_{\text{biosynthesis}}$ ($\sim$ 4 ‰). Heterotrophic bacteria will primarily reflect the values of the substrate carbon; however a $\sim$ 2 to 4 ‰ shift can occur during lipid biosynthesis (Pancost and Sininnghe Damsté, 2003, and references therein). $\delta^{13}C_{\text{mob-hopane}}$ is the value of the hopanoids derived from MOB. It is calculated from the $\delta^{13}C_{\text{methane}}$ minus the fractionation that occurs during carbon uptake by methanotrophs (0–30 ‰ ; Jahnke et al., 1999) minus the biosynthetic fraction during lipid synthesis ($\Delta^{13}C_{\text{biosynthesis}}$; $\sim$ 10 ‰). MOB can be significantly depleted in comparison to the source carbon they utilise (Whiticar, 1999); isotopic differences can be as large 30 ‰ (Jahnke et al., 1999). With little information available on the fractionation of hopanoids during their biosynthesis by MOB, we assumed a conservative value of 10 ‰ for our study. Four end-member values were calculated, taking into account maximum and minimum extremes for $\delta^{13}C_{\text{dip}}$ and $\delta^{13}C_{\text{hetero}}$ (Table 1). A threshold of 10 % was used arbitrarily to identify the point at which we considered MOB to be contributing to the diploptene signal.

4 Results

Early-winter ice-bubble surveys combined with bubble-trap measurements of ebullition flux and bubble methane concentration revealed that ebullition seeps occur with high density in the thermokarst zone (2.27 seeps m^2 and 4.2 seeps m^2 for Smith L.

and Ace L., respectively) compared to the rest of the lake (0.35 and 0.67 seeps m^2 for Smith L. and Ace L., respectively). Seep ebullition values in the thermokarst bays were 85 and 151 mg CH$_4$ m^{-2} d^{-1} for Smith L. and Ace L., respectively (Fig. 2). In the rest of lake (lake centre and non-thermokarst margins) seep ebullition was 6 and 20 mg CH$_4$ m^{-2} d^{-1} for Smith L. and Ace L., respectively. The δ^{13}C values for methane in bubbles collected from seeps in the thermokarst zones were −60.9‰ and −64.6‰ for Smith Lake and Ace L., respectively. At Smith L., the radiocarbon age of methane in ebullition bubbles collected adjacent to the margin was ∼ 2 ka, indicating a dominant Holocene carbon source (likely decomposing near-surface peat). No radiocarbon dates of methane were available at Ace L.

Diploptene was detected in all but one of the samples analysed (Table 2; Fig. 3). This sample was not part of further analysis. In the Ace L. thermokarst zone, diploptene values ranged from the lowest value for the whole dataset of −68.2‰ to −50.1‰. The most negative value was found at the greatest water depth (3.2 m) and was the only sample that does not lie within 1 standard deviation of the mean. However, another sample at the same depth was far less depleted (−50.1‰), which suggests the low δ^{13}C value is not explained by water depth. In Smith L., diploptene δ^{13}C values ranged from −56.8‰ to −38.8‰. Samples from the centre and edge of Smith L. ($n = 6$, $n = 3$ respectively) were compared and a Mann–Whitney U test applied (H0: diploptene δ^{13}C values are not different). The values for Smith L. indicates that the MOB proportional contributions to the total bacterial communities differed significantly between the two sample zones, values from the thermokarst zone of Smith L. being higher (−42.9 to −38.8‰) than those in the lake centre (−56.8 to −46.9‰).

Diploptene δ^{13}C values in the thermokarst zone of Ace L. are similar to those of the lake centre at Smith, and values from the Smith thermokarst zone are higher than both of these. Thermokarst zone diploptene δ^{13}C values at Ace Lake were more negative than those at Smith Lake by at least 10‰, despite methane δ^{13}C values being less than 5‰ different. However, the samples in the thermokarst zone of Ace L. and the

12171

centre of Smith L. ($n = 4$, $n = 6$ respectively) were not significantly different according to a Mann–Whitney U test.

The potential contributions of MOB, under different end-member assumptions, to the diploptene signal are shown in Table 3. The minimum and maximum possible contributions range from 19 to 85 %, 7 to 27 % and 19 to 63 % for Ace L. thermokarst zone, Smith L. thermokarst zone and Smith centre, respectively.

5 Discussion

5.1 Distribution of ebullition seeps

The spatial distribution of ebullition seeps at Ace L. and Smith L. adheres to the general pattern of seep occurrences as described in other studies, (Walter Anthony and Anthony, 2013), in that the highest density of methane ebullition seeps were found in the thermokarst zone.

5.2 The presence and spatial variability of MOB

The δ^{13}C values of diploptene ranged from −68.2‰ to −38.8 (Fig. 3), values similar to those that have been previously invoked as evidence for methanotrophy in lacustrine sediments (−64 to −55‰; Spooner et al., 1994; Naeher et al., 2014), marine sediments (−62 to −35‰; Freeman et al., 1994; Thiel et al., 2003) and in wetlands (−40 to −30‰ to van Winden et al., 2010; Zheng, 2014). Therefore, we conclude that diploptene δ^{13}C values are reflecting the presence of MOB bacteria in lake sediments. The lowest values in Ace L. are among the lowest reported for lacustrine (or other terrestrial), suggesting a relatively high degree of methanotrophy in those sites. In the thermokarst zone at Ace L., the diploptene values were highly variable but all suggested MO was occurring, and the fraction of diploptene derived from MOB was > 10 % even under the most conservative assumptions (Table 3).

12172

The results of the mixing model suggest that MOB can contribute anywhere between 7–83 % of the diploptene production across all sampled areas (Table 3). We note that there are some important caveats to using this mixing model. Crucially, diploptene is not derived from all bacteria nor even all methanotrophic bacteria (Rohmer et al., 1987). Nor does it likely occur in constant biomass-to-lipid ratios in those organisms from which it can derive, such that extrapolations from a diploptene mass balance to inferring bacterial biomass distributions should be done cautiously. They are best considered semi-quantitative. Nonetheless, a MOB contribution to total biomass of ~ 10 to 80 % is similar to that derived from other studies (11–80 %; Bastviken et al., 2003; Sundh et al., 2005; Kankaala et al., 2006). Regardless of absolute MOB estimates, our data show that the centre of Smith L. and the thermokarst zone at Ace L. have the highest proportion of MOB in the total bacterial biomass.

The data presented here allow us to develop, alongside other studies, models of methane production and emission pathways in thermokarst lakes. At Ace L., MOB biomass was high relative to other samples collected in this study and in the context of previous studies. Ace L. is a "yedoma-type" lake and has a high methane ebullition flux ($151 \, mg \, CH_4 \, m^{-2} \, d^{-1}$), likely derived from older (e.g. Pleistocene), deeper sediments in the talik bulb (Walter et al., 2008; Sepulveda-Jauregui, 2015). Given the coincidence of high bubble counts and high estimated MOB biomass, it could be assumed that the supply of dissolved methane and therefore MO is high in the thermokarst zone.

Ace L. appears to be representative of the enhancement model, whereby methane ebullition flux from bubble tubes increase the amount of methane diffusion from the sediments. In Ace L., and by extension other yedoma-type thermokarst lakes, where methane is produced in deep sediments the increased contact time with sediment (both over distance and time taken for bubbles to reach the sediment–water interface) may allow for increased methane diffusion in adjacent sediments. Alternatively, thermokarst erosion of yedoma-type permafrost is also known to supply nitrogen and phosphorus to lakes (Walter Anthony et al., 2014), enhancing primary production, which in turn can fuel methanogenesis and MO from contemporary (atmospheric) carbon (Martinez-Cruz

12173

et al., 2015). We cannot definitively distinguish between these alternatives since the carbon utilised by MOB observed in Ace L. could be derived from deep, ^{14}C-depleted methane and/or from shallow-sediment, contemporary methane.

Within the thermokarst zone at Smith L. the $\delta^{13}C$ values of diploptene were less variable (range: 10 ‰) than the Ace L. thermokarst zone (18 ‰) and the $\delta^{13}C$ values were overall more enriched (−42.9 to −38.8 ‰). In fact, the thermokarst zone in Smith L. had the lowest proportion of MOB for the entire dataset, with a MOB contribution to diploptene being equivocal for most of these samples. Conversely, samples from the centre of Smith had diploptene $\delta^{13}C$ values that were similar to those of the Ace thermokarst zone. The differences between the centre and the thermokarst zone could arise from alterations in the microbial community that manifest as different MOB expressions of hopanoids, for example, Smith L. thermokarst zone MOB might not be biosynthesising diploptene or its precursor. Alternatively, there may be differences in the balance of MO for energy vs. biomass production. Another explanation for the difference in $\delta^{13}C$ values, which could be validated through further investigation, could be due to differences in the methane production pathways as highlighted by Walter et al. (2008). The higher $\delta^{13}C$ values of diploptene could be due to more enriched methane formed through acetate fermentation. The most direct interpretation given the currently dataset, however, is that MOB are more abundant in the centre of the lake than at the thermokarst margin and, by extension, more MO is taking place in the lake centre. Given the pattern of high MO in the centre of Smith L. and less MO at the edge but more flux to atmosphere via ebullition, it seems that Smith methane dynamics are more akin to those of "clastic" lakes or other, non-thermokarst boreal lakes (e.g. Bastviken et al., 2004). The patterns at Smith L. also suggest that methane dynamics in the thermokarst zone follow the by-pass model in which methane ebullition is an independent process that interacts weakly with the lacustrine system.

Overall, the Smith thermokarst zone had lower methane ebullition rates ($85 \, mg \, CH_4 \, m^{-2} \, d^{-1}$) and less negative $\delta^{13}C$ of methane as measured from ebullition flux (−60.9 ‰) than Ace L. It is possible that this methane is not produced in the talik,

12174

but in near-surface sediments likely derived from peat slumping at the margin. This is supported by the late Holocene radiocarbon date of ebullition seep methane. The large size of the sediment blocks and the early stage of decomposition of the organic material that slump into the lake may mean there is less exposed substrate surface area and less methane production, as compared to yedoma-lake production from the fine-grained and more labile sediments. Production in shallower sediments (and often shallow water depths) means reduced partial pressure and faster release of bubbles from the sediment. Here, if bubble tubes initiate in shallower sediments (that are shallower than the talik bulb but deeper than the anoxic surface sediments) and the overall number, size and intensity of bubble tubes is reduced, then the connection between ebullition and diffusion could be decoupled. Whether there is a reliable connection between ebullition flux and high diffusion in the thermokarst zone is still to be determined, but the results of this novel but preliminary study highlight the need to continue research in this area.

5.3 Assessing past and current carbon cycling in thermokarst lakes

A crucial outcome of this study is the large variability seen in the δ^{13}C values of diploptene across small spatial distances. This is an important finding, as often whole lakes can be represented by a single sampling site in palaeoenvironmental studies. Such large fluctuations in δ^{13}C values in surface sediments, which were taken as replicates (e.g. repeat samples from the same zone within a lake), highlight the need for caution when interpreting shifts in δ^{13}C values through time (i.e., down a single sediment core).

While the differences in diploptene δ^{13}C values between chosen study zones discussed above are statistically significant, the sample number is small, and this topic could benefit from further sampling. There is a large degree of heterogeneity in the values in all three study areas. Interestingly, previous studies of MOB in lake sediments also show large variability in bacterial communities across small spatial extents (Kankaala et al., 2006). This could have implications for interpretation of not only

12175

biomarkers but also other geochemical records. For example, it is unclear how high spatial and temporal variability in MOB biomass affects the isotopic composition of consumers higher in the food web. The biological and geochemical connections between MOB and higher trophic organisms need to be better understood in order to interpret past methane emissions.

6 Conclusions

A primary aim of our research was to contribute towards the understanding of the links between methane production, transport and recycling in thermokarst lakes. Diploptene δ^{13}C values were used as a proxy for MO that could be linked to variations in methane supply via diffusion in thermokarst lakes. Diploptene was present in almost all samples and its δ^{13}C values were highly variable. A two-part mixing model highlighted potential variation in total MOB biomass with almost no MOB contributing to bacterial biomass in some samples but forming over half the total bacterial population in others. Like methane production, MO is highly complex, both in terms of its spatial distribution and in relation to the type of substrate available. A single model for thermokarst lakes is unlikely to capture all patterns present at both the inter-lake and intra-lake level. Thus, it is crucial that interpretation of diploptene δ^{13}C values (and other MO proxies) in palaeoenvironmental investigations take into lake type (e.g., yedoma or non-yedoma) and possible spatial heterogeneity in methane production pathways. Moreover, future work should examine localized spatial variability of MO within lakes and how spatial variation is integrated temporally, as this may critically affect observed down-core patterns of biomarkers and their isotopic signals. We conclude that diploptene biomarkers have considerable potential to help reconstruct patterns of methane cycling in lakes and, with certain caveats, particularly attention to context, past methane dynamics.

12176

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Table 1. Mixing model end member values and δ^{13}C values of the primary variables used to calculate the proportion of MOB at each sample point. $\delta^{13}C_{bulk}$ is the average bulk sediment value from each lake, ± indicates the standard deviation of the $\delta^{13}C_{bulk}$. MOB and heterotrophic bacteria have been assumed to have maximum levels of lipid biosynthesis occurring (10 and 4 ‰ respectively). $\delta^{13}C_{mob-hopane\_min}$ is the estimated minimum stable isotope value given the δ^{13}C value of methane at each lake and the maximum potential fractionation of carbon by MOB. $\delta^{13}C_{mob-hopane\_max}$ is the estimated value of MOB with no fractionation during assimilation. $\delta^{13}C_{hetero-hopane\_max}$ is the maximum estimated stable isotope value of heterotrophic bacteria if no fractionation is occurring during assimilation and the bulk sediment is +1.0 standard deviation (S.D.) from the mean at each lake. $\delta^{13}C_{hetero-hopane\_min}$ represents the minimum value for heterotrophic hopanes given maximum possible fractionation during assimilation and if bulk sediment is −1.0 S.D from the mean.

	$\delta^{13}C_{bulk}$ ±	(‰)	$\delta^{13}C_{mob-hopane\_min}$ (‰)	$\delta^{13}C_{mob-hopane\_max}$ (‰)	$\delta^{13}C_{hetero-hopane\_min}$ (‰)	$\delta^{13}C_{hetero-hopajne\_max}$
Ace	−30.8	2.1	−104.6	−74.6	−36.9	−32.7
Smith	−29.3	0.8	−100.9	−70.9	−34.1	−32.5

Table 2. δ^{13}C values of diploptene at the study sites.

		Sample Number	$\delta^{13}C_{dip}$ (‰)	Standard Deviation (SD)
Ace				
	TK zone	a1	−50.1	1.5
		a2	−58.5	2.0
		a3	−53.1	0.4
		a4	−68.2	0.1
Smith				
	Centre	1	−51.4	2.7
		2	−48.3	0.0
		3	−56.8	N/A
		4	−49.2	1.0
		5	−46.9	1.8
		6	−48.0	0.1
	TK zone	7	−38.8	0.3
		8	−40.9	0.2
		9	−42.9	0.1
		10	N/A	N/A

Table 3. Estimated contribution of MOB to the diploptene signal. Calculations assume fractionation due to biosynthesis of 10‰ for MOB and 4‰ for heterotrophic bacteria. f_{mob_min} was calculated assuming the highest fractionation for both MOB and heterotrophs (30 and 4‰ respectively). f_{mob_max} assumes no fractionation during assimilation. $f_{mob\_average}$ was calculated using average $\delta^{13}C$ values for $\delta^{13}C_{mob\text{-}hopane}$ and $\delta^{13}C_{hetero\text{-}hopane}$.

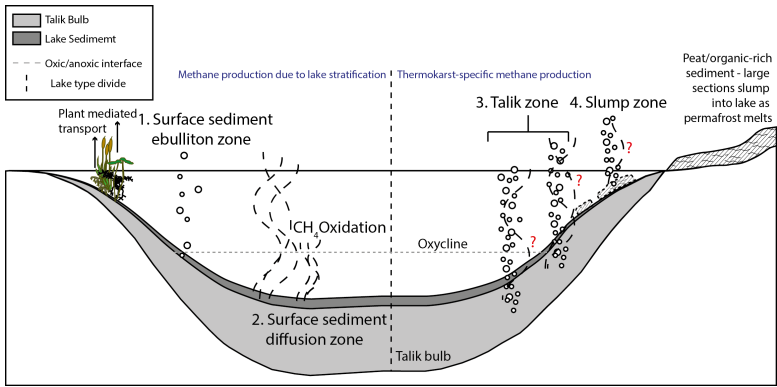|		Sample Number	f_{mob_min}	f_{mob_max}	$f_{mob\_average}$
Ace					
	TK zone	a1	0.19	0.42	0.28
		a2	0.32	0.62	0.43
		a3	0.24	0.49	0.33
		a4	0.46	0.85	0.61
Smith					
	Centre	1	0.26	0.49	0.34
		2	0.21	0.41	0.28
		3	0.34	0.63	0.45
		4	0.23	0.44	0.30
		5	0.19	0.37	0.26
		6	0.21	0.40	0.28
	TK zone	7	0.07	0.17	0.11
		8	0.10	0.22	0.14
		9	0.13	0.27	0.18

Figure 1. Illustration of methane production zones and emission pathways in lakes alongside thermokarst-specific zones and pathways. (1) Surface sediment ebullition zone. Methane that is produced in the anoxic surface sediments is released via ebullition, usually near the margins (Bastviken et al., 2004). (2) Surface sediment diffusion zone. Methane is produced in the anoxic surface sediments and diffuses in the sediments above and into the water column. Some of this methane will reach the water surface–air interface but a large amount is likely to be oxidised by MOB (Kankaala et al., 2006). This process is common in many lakes also. (3) Talik zone. Methane is produced in the deeper talik sediments underneath the lake and is released via ebullition seeps (Walter et al., 2008). Often this is a higher flux and is more constant than surface sediment ebullition. This production zone and pathway is a thermokarst-specific process. (4) Slump zone. Methane production in the surface sediments is increased due to the introduction of large volumes of slumped sediments. This methane is also released via ebullition seeps. Often, the flux from these ebullition seeps is higher than surface sediment ebullition but not as high as talik ebullition. This process might occur in any lakes that have dynamic margins and high erosion rates; however, it is likely that this process is most common in thermokarst lakes due to the melting of permafrost, so it is termed thermokarst-specific. Red?'s indicate where methane diffusion from the sediments has not been studied in detail.
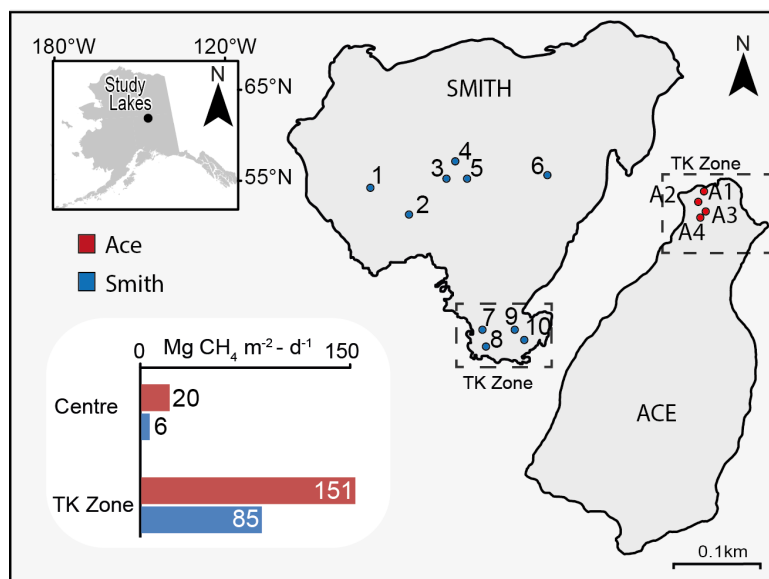
Figure 2. Locations of the study lakes in Alaska and the sample points within each lake. The red (Ace L.) and blue (Smith L.) bars indicate the flux values as measured at an individual ebullition seep within a given area of the lake.
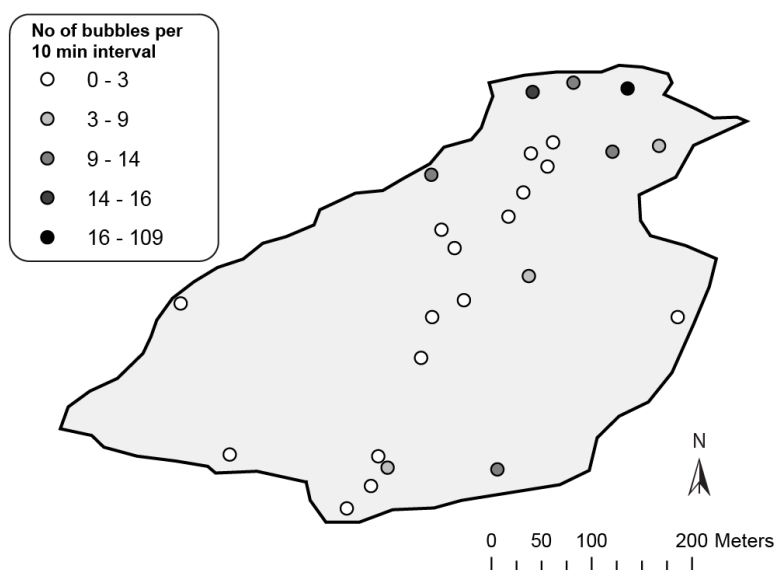
Figure 3. Bubble counts at Ace Lake. A tally of all bubbles that broke at the water surface within a 2 m radius of the sample location. The thermokarst zone is found at the top of the lake and has the highest bubble counts.

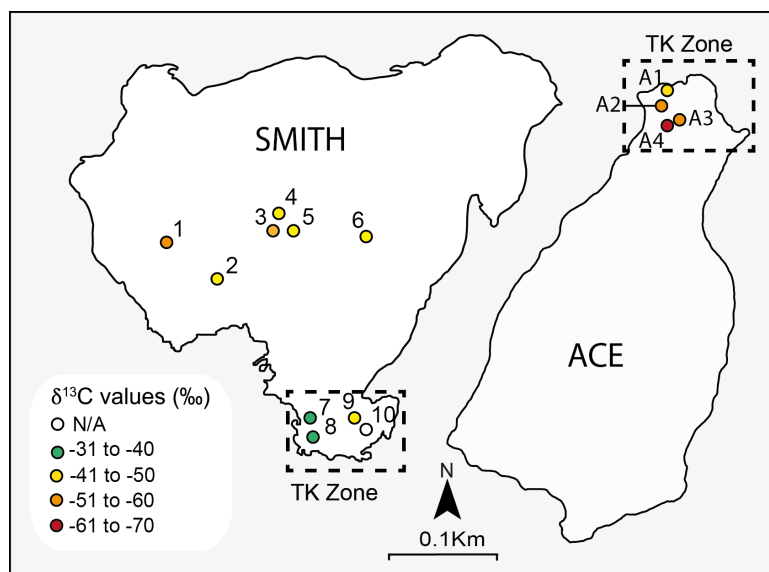**Figure 4.** Diploptene δ^{13}C values at Smith Lake and Ace Lake. In general the most depleted values are found in Ace L. and the centre of Smith L. The thermokarst zone at Smith L. has the least depleted values for the whole dataset.