alterations make it clear to the reader that the results are complicated and they reveal the potential variability which can be found in contemporary sediments even in a small lake.

GC3: I think this manuscript can be published, as long as the authors are clear that this is a very preliminary study, which basically tells us that with more research, δ13Cdiploptene might become useful as a biomarker for informing methane oxidation history in lake sediments. But for now, we don't know enough to reliably apply it.

Response 3: We very much agree with this and this is a fundamental point that the manuscript is trying to make. We have included changes to the abstract and the conclusion which should make these points much clearer.

Sentences altered/added:

The diploptene $\delta^{13}C$ values highlight strong within-lake variability but presently, there is no clear pattern in this variability that can be linked to thermokarst specific methane emissions.

This study, whilst preliminary, highlights the need for further research and implies that at this stage, single-value, down-core records of hopanoid isotopic signatures are unlikely to be secure indicators of changing methane flux at the whole-lake scale.

However the results were highly variable and suggest that, 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, and as the data stand, there is a large amount of variability which cannot be linked to specific types of methane production.

We conclude that given the current data, further research should be completed in order to understand the variability in d13c diploptene values prior to utilisation of this method for the reconstruction of methane cycling in lacustrine systems.

Specific comments (SC)

SC1: Diploptene is mispelled TWICE in the abstract. 'Diplotene' is something completely different (and is not a chemical).

R1: We have fixed these grammatical errors.

SC2: In the abstract: 'Using δ13C-diploptene 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

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Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 15:50:15

I am not sure the referee was expecting you to become negative about your dataset. I think part of the problem is related to the link between ebullition rate vs diffusion rate AND between MO and CH_4 production rate, and not so much between diploptene index and MO. See below comment in ms

and type of organic carbon) and bathymetry as it relates to varying oxycline depths and changing pressure gradients.'

Ok...but as noted in the manuscript, there was no radiocarbon dating in one of the lakes. So it seems that the suggestion of age differences is premature

R2: We have removed the reference to chronology in the abstract and altered the wording.

SC3: Section 5.3: "A crucial outcome of this study is the large variability seen in the δ13C 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."

I agree with this! But it undermines some of the conclusions of the manuscript, especially the last statement of the conclusions: "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."

Isn't it more true that this study raises MANY cautions that must be resolved before δ13C-diploptene values can be used to 'reconstruct patterns of methane cycling and past methane dynamics'?

I don't see how the results in this study do much more than show that sometimes the δ13C-diploptene values make sense with current observations of methane ebullition and methane oxidizing bacteria biomass, and sometimes they don't (e.g. Figure 4)

R3: We agree that this final sentence in the conclusions was out of place and did not fit with what the data are showing. We have replaced this sentence with one that highlights that more work should be done.

New sentence:

We conclude that given the current data, further research should be completed in order to understand the variability in d13c diploptene values prior to utilisation of this method for the reconstruction of methane cycling in lacustrine systems.

Minor Comments (MC)

MC1: 'The connections between methane production..." This sentence should be split into two sentences, probably after 'not well understood'

Page : 3

Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-15 14:09:18
and correct syntax

SC3: 3.2 Methane monitoring: the authors mentioned methane δ13C and δD, but didn't show/discuss them in the paper.

R3: The mention of δD has been removed from the manuscript. The δ13C we refer to can be found in table 1 and is used as part of the mixing model.

SC4: 3.5 Mass balance equation: ". . ..δ13C hetero_hopane is the δ13C value of the hopanoids derived from heterotrophic bacteria. . .", So please specify which hopanoids in the paper because a lot of hopanoids are derived from bacteria

R4: From this comment, we can see that the reference to other types of hopanoids is confusing. We have altered the wording of the mixing model to better reflect what we were trying to show. We have added a sentence in the introduction to help convey the theory behind the mixing model.

The mixing model is trying to understand the contribution of MOB to the diploptene signal, where diploptene is derived from both MOB and heterotrophic bacteria. Here, shifts towards more negative d13c values would suggest a greater contribution of MOB to the diploptene signal.

Added sentences:

In particular, the compound diploptene (17 β(H), 21 β(H)-hop-22 (29)-ene), is a hopanoid hydrocarbon derived from a range of bacterial sources. however due to the utilisation of methane as a carbon source, the d13c values of diploptene derived from MOB will be more negative than if it were derived from other heterotrophic bacteria which utilise organic carbon from vegetation.

The δ^{13}C values of diploptene derived from heterotrophic bacteria will primarily reflect the substrate carbon which in this instance will be organic material and not methane. These values are therefore unlikely to vary; however a ~2 to 4‰ shift can occur during lipid biosynthesis (Pancost and Sininnghe Damsté 2003, and references therein).

SC5: Results section of Line 24-25(P12171): '. . .. . .Diploptene δ13C values in the thermokarst zone of Ace L. are similar to those of the lake centre at Smith,. . ...', I couldn't see they are similar.

R5: we have removed this sentence

SC6: Line 14 in the 5.2 section, it is fig3 or fig.4?

Nombre : 1 | Auteur : | Sujet : Commentaire sur le texte | Date : 2015-12-18 15:52:35

table 2

R6: This should be figure 4, we have amended this.

SC7: 5.2 section and Table 3: It is also not very clear that MOB biomass has large variations across the small distances. For example, at the TK zone of Ace Lake, sample a3 and a4 are close, but the difference of MOB biomass is around 30%. If it is because of microbial community, so give more evidence.

Referee #3

SC1: In the abstract diploptene is misspelled twice.

R1: We have fixed these grammatical errors.

SC2: Page 12163 Line 4, What is a "bight"

R2: We have included a brief definition of a bight in the text. Briefly, a bight is a curve in the coastline or a bay formed from such a curve.

SC3: Page 12164 Line 2-4, "potential confounding factor. . ." this seems potential pretty important, what impact could this have on your results.

R3: This factor is discussed in section 5.2, however we have added more detail in the introduction.

Sentence added:

Methane production and oxidation that occurs in the near-surface sediments will represent a background level which is likely to be found in many contemporary lake settings and the amount should be lower than that derived from thermokarst specific sources. We might expect some level of depletion in $\delta^{13}C$ values due to near-surface production but crucially, if $\delta^{13}C$ values of diploptene are to be used as a proxy for past methane production, we would expect thermokarst specific methane production that is being oxidised would have much lower $\delta^{13}C$ values than background methane oxidation.

SC4: Introduction, it is not clear exactly what patterns you would you expect to see in diploptene δ13C under the scenarios discussed.

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Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 15:53:41
has this been addressed?

Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 15:57:24
specify a background level of what

R4: We have added a number of sentences which make it clearer what patterns we would expect to see.

Sentences added:

In particular, the compound diploptene (17 β(H), 21 β(H)-hop-22 (29)-ene), is a hopanoid hydrocarbon derived from a range of bacterial sources. However due to the utilisation of methane as a carbon source, the d13c values of diploptene derived from MOB will be more negative than if it were derived from other heterotrophic bacteria which utilise organic carbon from vegetation.

Therefore if MOB are present in the sediments of thermokarst lakes, we would expect to see depleted $\delta^{13}C$ values of diploptene.

We might expect some level of depletion in $\delta^{13}C$ values due to near-surface production but crucially, if $\delta^{13}C$ values of diploptene are to be used as a proxy for past methane production, we would expect thermokarst specific methane production that is being oxidised would have much lower $\delta^{13}C$ values than background methane oxidation.

SC5: Page 12168, Line 5, Any particular reason for using the 1-2cm sediment slice?

R5: The 0-1cm sediment slice was more variable is sample size due to the sediment-water interface, therefore the 1-2cm slice is still well oxygenated and was more likely to represent the same level across all samples.

SC6: Page 12167, Line 25, Don't include the δD analytical error if you don't include any δD data.

R6: The mention of δD has been removed from the manuscript.

SC7: Page 12170 Line 11, You give a potential range of 0-30‰ what value did you use, is this the 10‰ you discuss earlier, please clarify.

R7: We have added sentences to clarify what we did. The results are presented as a range which incorporate both the minimum and maximum possible fractionation factor, therefore $d13c_{MOB\_dip\_min}$ will represent the lowest possible value given maximum fractionation (30‰).

Sentences added:

In order to incorporate this large range, we used both the minimum and maximum value of fractionation (0 and 30‰) to show different scenarios rather than assuming a single value. This should also cover any potential variation due to differing $\delta^{13}C_{methane.}$ Therefore the

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capital letter

Nombre : 2	Auteur :	Sujet : Commentaire sur le texte	Date : 2015-12-18 15:58:47

specify production of what

Nombre : 3	Auteur :	Sujet : Commentaire sur le texte	Date : 2015-12-16 11:14:46

was this tested?

equation was calculated twice, once using $\delta^{13}C_{mob\_dip\_min}$ and once using $\delta^{13}C_{mob\_dip\_max}$.

SC8: Overall the calculation of diploptene δ13C seems pretty vague with a lot of estimates, this is ok, isotopes can be messy, but the discussion of these choices and the variation/uncertainty they introduce could be more clearly discussed, especially give the high variability and inconsistency of your results and the claims that this method could be used to do historical reconstructions.

R8: We are unsure if the reviewer is referring to the d13c values we have of the diploptene from the sediments or the d13c values inferred to calculate MOB concentrations. If referring to the latter, then we agree that they are messy and deliberately vague as we did not want the estimates to seem more robust than we can actually calculate. We have included further sentences to clarify the purpose of the mixing model.

We have also pointed out more clearly that this area of research needs much more development before it could be used for reconstructions.

Sentences added:

By developing this mixing model and considering, in more detail, the potential end member values for the $\delta^{13}C$ values of diploptene derived from different sources (MOB and other heterotrophic bacteria) we can get a semi-quantitative idea of the distribution patterns of MOB across the samples.

These estimates have a large degree of uncertainty associated with them and we note that there are some important caveats to using this mixing model.

SC9: Line 12171 Line 6, How many bubbles were sampled for δ13C, there are no error values listed, which seems to suggest only a single sample was analyzed at each site. If that is the case, there is not much you can infer from this one number; especially considering how your diploptene δ13C data shows just how spatially variable δ13C is in this system.

Nombre : 1	Auteur :	Sujet : Commentaire sur le texte	Date : 2015-12-16 11:23:29

replace by estimation

R9: The value from Ace Lake represents an average from across 5 seep locations in the thermokarst zone whilst the value from Smith Lake is taken from a single seep. We agree that the number of samples should be increased but in this instance, no more samples can be taken. These values have been used for the mixing model and we agree that it is likely that these values will be variable, however, we hoped that by using a minimum and maximum value for fractionation we would have incorporated a large amount of variation. We have included a sentence in the mixing model section which discusses this further.

Added sentences:

The $\delta^{13}C_{methane}$ is the measured value of methane captured at seep locations in the thermokarst zones at each lake. As the value is based on a limited number of data (n1 and n5 for Smith L. and Ace L. respectively), it is likely there will be more variation than is seen in the model. Furthermore 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). In order to incorporate this large range, we used both the minimum and maximum value of fractionation (0 and 30‰) to show different scenarios rather than assuming a single value. This should also cover any potential variation due to differing $\delta^{13}C_{methane.}$ Therefore the equation was calculated twice, once using $\delta^{13}C_{mob\_dip\_min}$ and once using $\delta^{13}C_{mob\_dip\_max.}$

SC10: Methods: Sample size, replication, sampling location information needs to be clearly covered in the methods section. This information needs to be included for all analyses, not just diploptene δ13C, although I couldn't even find sample size information for diploptene δ13C in the methods section (it is mentioned later in te manuscript).

R10: we have included a table which shows the sample weights and made reference to figure 2 which shows the sampling locations for the sediment cores.

Table included:

Sample No	Sample size (dry g)

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I do not think reviewer is asking to provide all the dry weight numbers, but rather a range.

On the other hand, a clarification of the number of replicates for **all variables** presented in the ms is missing (diploptene 13C in CH4, ebullition rates, bubble counts, 14C…)

Smith		
	1	0.2596
	2	0.2206
	3	0.3584
	4	0.1486
	5	0.1942
	6	0.5654
	7	0.3841
	8	0.2024
	9	0.3386
	10	0.2185
Ace		
	a1	1.3427
	a2	0.6812
	a3	0.5758
	a4	0.808

SC11: It looks like Ace lake was only sampled in the TK zone whereas Smith Lake was also sampled away from the TK zone. This is unfortunate, since it really limits the ability to distinguish potential impacts of thermokarst activity from other spatial differences within/between lakes.

R11: We agree that this is a shame. The centre of Ace L. is much deeper than at Smith L. (~9m) and we wanted to try to reduce the number of other potential factors which could influence the d13c values. Furthermore, due to the limited number of samples which could be run, we felt it would be more beneficial to get repeat samples from within zones. We have included a sentence in the methods to point this out.

Sentence added:

In order to remove water depth as a confounding variable and to increase the number of replications in each zone, Ace L. was not sampled as it was much deeper than Smith L. centre (~9m).

Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:02:35

I guess you rather mean "middle (or deepest part of the lake), located away from the thermokarstic zone, was not sampled"?
And why depth would be a confounding effect, because of the anoxia level? please be more explicit.

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~~ T~~t~~his has implications for any attempts to reconstruct methane production from sedimentary archives. ~~We assessed m~~ Methane oxidising bacteria were used to represent methane oxidation ~~ation 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 ^{13}C-depleted diploptene was found in the area of highest methane ebullition emissions in Ace Lake ($\delta^{13}C$ diploptene values between -68.2 and -50.1‰), suggesting a ~~positive~~ potential 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 $\delta^{13}C$-diploptene 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 the level of methane diffusing from the sediments, the source area of methane production (e.g. surface versus deep sediments~~age and type of organic carbon~~) and bathymetry as it relates to varying oxycline depths and changing pressure gradients, although these theories need to be tested. ~~As a result, methane oxidation is highly lake-dependent~~. The diploptene $\delta^{13}C$ values ~~also~~ highlight strong within-lake variability but presently, there is no clear pattern in this variability that can be linked to thermokarst specific methane emissions~~,~~. This study, whilst preliminary, highlights the need for further research and impl~~ying~~ies that at this stage, single-value, down-core records of hopanoid isotopic signatures are ~~not~~ unlikely to be secure indicators of changing methane flux at the whole-lake scale.

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Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:04:35
this is indirectly inferred from diploptene and i think it would be better to say "Diploptene, used as a proxy for MOB, …"

Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:05:16
methane oxidation **level**

Nombre : 3 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:06:20
I suggest to use emission rate (here and below). I think emission is a term for the dynamic, and when you speak about a rate you should mention it (emission rate via ebullition, or ebullition rate)

Nombre : 4 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:06:44
unnecessary synonym

Nombre : 5 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:07:19
before providing results (and even before previous sentence to better understand MOB use), we need a sentence explaining what is diploptene (used as a biomarker)

Nombre : 6 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:09:37
but we do not have other area in this lake right? maybe for Smith L. 42 is relatively a high value? So maybe this index can only be used for one specific lake i.e. relative to itself and along a sediment core?
You need to specify what is this region of highest ebullition rate for this lake

Nombre : 7 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:09:48
appears??

Nombre : 8 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:10:00
hypotheses?

Nombre : 9 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:10:58
I suggest to rephrase this, maybe something like "but presently, the patterns are not clear enough to allow using this proxy as an index of CH_4 emsission rate"

Nombre : 10 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:14:06
to my point of view, this is not acceptable to say a sentence like this. If a work is to be published, it needs to be more than preliminary and without any patterns. It is OK to present a limited data set if the results show a promising trend. But if you do not believe more than that in the potential of this index, I have a hard time to convince myself that the work is worth publishing. I think you need to convince the readers of the potential, with an explicit acknowledgement of the limitations of your data set. I think you need to better sell your work.

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 lake 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 (Figure 1). Anoxia is caused by oxygen depletion associated with microbial decomposition of organic matter. 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

Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:14:50
6 pages of introduction is rather long, but I assume the subject is complex because of the many steps (see below comments on these many steps)

Nombre : 2 Auteur : Sujet : Barrer Date : 2015-12-16 13:48:46

Nombre : 3 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:15:33
what proxy are you talking about??
I suggest to eliminate this part of sentence, or make it as a separate sentence where you explicitely mention what proxy you are referring to. I think you also need to mention what knowledge is needed to improve our understanding of "CH4 dynamics": the link between production and consumption? a correct assessment of diffusion vs ebullition? the link between microbial activity and OC lability? the controlling factors on production/consumption/emission? etc. At least mention a few.

Nombre : 4 Auteur : Sujet : Barrer Date : 2015-12-16 14:14:48

Nombre : 5 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:15:48
I don't see why **rapid** burial is more prone to generate anoxia

Nombre : 6 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 14:33:43
tautology

Nombre : 7 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 14:58:45
here you are talking about production right? make sure to use the correct term throughout the ms (emission - or better emission rate - refeers to the flux at the air-water interface)

typically of Holocene age, but may be older (Figure 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., 2014; 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 (Figure 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.

Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 14:59:55
or higher quantity?

Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 15:07:12
see comment in ref list

Nombre : 3 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:17:51
maybe specify that this is the case for yedoma lakes, or is it also on non-yedoma?

Nombre : 4 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:18:19
should you specify "yedoma thermokarst lakes"? cause in non-Yedoma thk lakes thaw bulb might exist but is concerning a shallower layer of organic sediment right?

Nombre : 5 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:19:22
what do you mean by a lake cumulating Holocene-aged OC? C burial after the lake inception or prior to its inception??

Nombre : 6 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:20:29
the logical link between these 2 sentences is huge and not evident from your work. I do not think you have data allowing to establish about diffusion vs ebullition, nor specifically concerning diffusive flux. Placing what you have done right after this sentence underlining the gaps generates an expectation to the readers that you will provide data on this.
And anyway, I think this last sentence about what you have done should better fit at the very end of Introduction

.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). Methane oxidation 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) methane oxidation 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 (9m) near-shore bight (a curved bay) 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 the 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

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Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 15:37:05
this sub-section also explore the link between MO and flux (or should you say MO and production?)

Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 15:18:52
There is no point in having a section numbered 1.1 if there is no more sub-sections in the Introduction

Nombre : 3 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:21:34
you seeem to imply that these 2 studies have measured **anaerobic** methanotrophy, but I don't think its right. Even if the lakes studied were stratified 1- it does not mean hypolimnion was anoxic, and 2- it does not mean MO was measured in anoxic zones of the lake (but please double check).

Nombre : 4 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 15:38:36
or CH4 production? (i.e. the link between production and consumption by bacterial oxidation)

Nombre : 5 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 15:39:25
??

Nombre : 6 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 16:05:44
in this paragraph, 2 concepts are mixed, confusing the logical flow or your reasoning: the link between ebullition and diffusion, and between production and oxidation. This will prepare better the next step which is a link between ebullition and MO, even if oxidation is not concerning CH4 escaping through ebullition...

Nombre : 7 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 16:24:35
it is not clear in this sentence if these studies have demonstrated a link between the production RATE and the oxidation RATE. I am assuming you want to be able to rely on a significant correlation between MPr and MOx to use your diploptene proxy of MOx to estimate past MPr… You really need to make this clearer otherwise your reasoning cannot support your *weak* index (unclear relation between ebullition/diffusion flux and diploptene in surface sediment).
By the way, the weakness of your index might also come from too few flux measurements, as discrete flux (n=1 or n=5 is still few) does not compare well with the time span of your diploptene signature in surface sediment (days?, season? year?). I think this should also be addressed.

Nombre : 8 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 15:54:34
rewrite (syntax problem cf the rest of sentence)
Is this referring to Martens and Klump (comparing shallow and deep marine sites? or it is referring to previous citations???

Nombre : 9 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 16:00:34
is this important to mention anyway?? (that it's curved, as long as we know it's near-shore and shallow

Nombre : 10 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 15:53:01
this whole sentence could be so much simpler: Martens and Klump demonstrated that ebullition rate is correlated to diffusive rate?

Nombre : 11 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:06:39
or CH4 ebullition rate from seeps? or number of seeps per square meter?

Nombre : 12 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 16:08:19
measured how? (briefly) MOB DNA? this is unclear cf the mention of methanotroph communities in next sentence

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.

[1]In contrast, based on $\delta^{13}C$ 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 [2]no difference between thermokarst-zone and lake-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. Methane production and oxidation that occurs in the near-surface sediments will represent a background level which is found in many contemporary lake settings however the [3]amount should be lower than that derived from thermokarst specific sources. We might expect some level of depletion in $\delta^{13}C$ values due to near-surface production but crucially, if [4]$\delta^{13}C$ values of diploptene are to be used as a proxy [5]for past methane production, we would expect that if thermokarst specific methane production is being oxidised, this would have much lower $\delta^{13}C$ values than even background MO.

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 $\delta^{13}C$ values (usually -8~~5~~0 to -~~8~~50‰, Whiticar, 1999), depending on the methane production pathway and substrate availability. These depleted $\delta^{13}C$ values can be traced through the food web, for example, in low-level heterotrophs such as invertebrates. Previous studies have linked depletion in the $\delta^{13}C$ 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 (non-biting midge) taxa utilise MOB as a food source within lakes

Nombre : 1	Auteur :	Sujet : Commentaire sur le texte	Date : 2015-12-21 10:07:14

why in contrast?

Nombre : 2	Auteur :	Sujet : Commentaire sur le texte	Date : 2015-12-16 16:19:31

it is unclear what difference you are speaking about: no difference between the 2 zones in terms of the diffusion of deep-sourced CH_4? i.e. similarly low?

Nombre : 3	Auteur :	Sujet : Commentaire sur le texte	Date : 2015-12-16 16:20:29

of both production and oxidation?

Nombre : 4	Auteur :	Sujet : Commentaire sur le texte	Date : 2015-12-21 10:07:58

You have not introduced yet what is diploptene (only done in next paragraph) so this sentence needs to be adjusted.

Nombre : 5	Auteur :	Sujet : Commentaire sur le texte	Date : 2015-12-16 16:24:07

I think you also need to clarify the difference between past CH_4 prodcution and present-day production from OC deposited in the past (old OC)

(Deines et al., 2007; van Hardenbroek et al., 2010). In thermokarst lakes, depleted $\delta^{13}C$ values in larvae and fossil head capsules have been linked to increased methane flux (van Hardenbroek et al., 2012). Wooller et al. (2012) also interpret negative shifts in $\delta^{13}C$ 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), is a hopanoid hydrocarbon derived from a range of bacterial sources. ~~,~~ However due to the utilisation of methane as a carbon source, the $\delta^{13}C$ values of diploptene derived from MOB will be more negative than if it were derived from other heterotrophic bacteria which utilise organic carbon from vegetation. In marine sediments, diploptene has been identified as a methanotrophic biomarker via ~~low~~ negative $\delta^{13}C$ values ~~in marine sediments and~~ as well as in microbial mats associated with methane seeps (Elvert et al., 2001b; Pancost et al., 2000a, 2000b) ~~as well as~~and 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). Therefore if MOB are present in the sediments of thermokarst lakes, we would expect to see depleted $\delta^{13}C$ values of diploptene.

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 MOB 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 $\delta^{13}C$ values of diploptene were used firstly to establish if MO was occurring at levels detectable by biomarkers, and secondly to assess the degree of

Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 16:46:59
why "however"?

Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 16:48:47
from any detritus and OM? (could also be from plankton and from DOM)

Nombre : 3 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:11:04
plus, it also depends on the relationship between production and oxidation; this 2-level dependency will affect any attempts to detect links between diploptene and production, and I think this could be discussed explicitely (only once in intro however).

Nombre : 4 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:13:19
I am not convinced that you investigate these issues! 1- you do not have diffusive rate data, 2- you do not have data to investigate the "sensitivity" to changing CH4 "supply"; Do you? what does supply means? This is confusing. Make sure you distinguished between what you are doing with this ms and what questions are surrounding the problematic, which is fine to mention in intro.

Nombre : 5 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:15:48
in? associated to?

Nombre : 6 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 16:58:38
this should be mentionned in the abstract, to value your contribution (i.e. not only underlining the absence of a clear relationship with emission rates, which is several steps ahead)

MO observed in areas characterized by different modes of methane production and transport to the atmosphere.

2 Regional context & 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 (Figure 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; figure 2) is a shallow (≤4 m), productive lake located in Interior Alaska. It has a gentle bathymetric profile with average water depths between 1-3m. 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 ~~(KLD, personal observation)~~. The lake likely originated by thermokarst processes (Alexander and Barsdate, 1971); comparisons of lake shorelines between the 1950s and today suggest that segments of the southern and western margins have been ~~actively thermokarsting~~ actively thawing and eroding 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°51'45.49N, 147°56'05.69W) 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).

Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-17 10:17:33
provide trophic status like you do for Ace

Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-17 10:16:32
maybe not the best qualificative for this characteristic

Nombre : 3 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-17 10:18:10
mention if algal blooms are present like you do for Smith

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 (figure 2). We obtained surface sediment cores well within the zone boundaries and as close to observed ebullition seep locations as possible (figure 2). 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. In order to remove water depth as a confounding variable and to increase the number of replications in each zone, Ace L. was not sampled as it was much deeper than Smith L. centre (~9m).

3.2 Methane monitoring

Ebullition gas samples were collected from seep locations in the thermokarst zone (n1 and n5 for Smith L, and Ace L. respectively) 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 $\delta^{13}C_{CH4}$, using a Finnegan Mat Delta V~~, and δD_{CH4} 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 $^{13}C/^{12}C$ 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~~

Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-17 10:18:25
sampling?

Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 15:45:14
I think you used area or zone, please homogenize terminology for clarity purpose

Nombre : 3 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:23:34
bubble counts seems to be illustrated in figure 3, or maybe to clarify, indicate how the 3 measurements relate (ice-bubble survey, summertime bubble counts and bubble-trap flux) and present the relationship in results and discussion. Now we need to work hard to see how consistent this is.

Nombre : 4 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:24:24
I assume this was not the motivation for NOT doing it (logistical constraints?), and it would have been interesting to see if there is a bias or not, comparing diploptene...

Nombre : 5 Auteur : Sujet : Texte inséré Date : 2015-12-17 10:34:27
at its deepest site

Nombre : 6 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:24:54
what about bubble-count approach?? how is this exploited vs bubble traps with GC ans SI? Indicate when and if in parallel to bubble traps...

Nombre : 7 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-17 10:48:33
mention when (date, period of the year)

Nombre : 8 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:25:25
but in the result section, we find out that 14C is not available for Ace L.

~~and~~ standard~~s~~ refers to the Vienna Pee Dee Belemnite (VPDB) ~~and Vienna Standard Mean Ocean Water (VSMOW), respectively~~. The analytical error~~s~~ of the stable isotopic analysies was ~~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) x 100, 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 0-5cm sequence was extruded at 1-cm resolution and retained for analysis; the 1-2 cm slice was subsampled for biomarker analysis. Sample sizes can be found in Table 1. ~~-~~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 0.05M 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 minutes and then centrifuged at 3000 rpm for 5 minutes. 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 minutes and then centrifuged at 3000 rpm for 5 minutes. 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 10ml 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. Silica gel columns (again, 0.5 g, 60 Å particle size) were prepared and conditioned with 4 ml of the recently prepared $CHCl_3$ sat

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Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:26:10

see comment above in response letter and below besides the table

Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-17 10:42:35

indicate extraction of what (lipids)

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_2O_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 $\delta^{13}C$ 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 rate; 130-300 °C at 4 °C/min; 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 δ13C values were expressed against the standard VPDB. All measurements were performed in duplicate.

3.5 Mass Balance equation

A carbon isotopic mass balance equation (Equation 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. By developing this mixing model and considering in more detail the potential end member values for the $\delta^{13}C$ values of diploptene derived from different sources (MOB and other heterotrophic bacteria) we can get a semi-quantitative idea of the distribution patterns of MOB across the samples.

The resulting end member values are given in table 2~~1~~. The equation is as follows:

$$f_{mob} = \frac{\delta^{13}C_{dip\_sample} - \delta^{13}C_{hetero\_dip}}{\delta^{13}C_{mob\_dip} - \delta^{13}C_{hetero\_dip}} \qquad (1)$$

f_{mob} is the fraction of diploptene generated by MOB and $\delta^{13}C_{dip\_sample}$ is the stable carbon isotopic composition of diploptene in a given sample. $\delta^{13}C_{hetero\_dip}$ is the inferred $\delta^{13}C$

Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-17 10:43:08

explain why

value of ~~the hopanoids~~ diploptene~~-~~ if it were derived solely from heterotrophic bacteria, the inferred other primary source of this hopane ~~hopanoids~~ in this setting. ~~, and~~ It is expressed as the $\delta^{13}C_{bacterial\ biomass} - \Delta^{13}C_{biosynthesis}$ (~4‰). The $\delta^{13}C$ values of diploptene derived from ~~H~~heterotrophic bacteria will primarily reflect the ~~values of the~~ substrate carbon which in this instance will be organic material and not methane. These values are therefore unlikely to vary;~~;~~ however a ~2 to 4‰ shift can occur during lipid biosynthesis (Pancost and Sininnghe Damsté 2003, and references therein). $\delta^{13}C_{mob\text{-}hopane\text{-}dip}$ is the likely value of the diploptene ~~hopanoids~~ if it were derived solely from MOB. It is calculated from the $\delta^{13}C_{methane}$ minus the fractionation that occurs during carbon uptake by methanotrophs (0-30‰; Jahnke et al., 1999) minus the biosynthetic fraction during lipid synthesis ($\Delta^{13}C_{biosynthsis}$; ~10‰). The $\delta^{13}C_{methane}$ is the measured value of methane captured at seep locations in the thermokarst zones at each lake. As the value is based on a limited number of data (n1 and n5 for Smith L. and Ace L. respectively), it is likely there will be more variation than is seen in the model. Furthermore 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). In order to incorporate this large range, we used both the minimum and maximum value of fractionation (0 and 30‰) to show different scenarios rather than assuming a single value. This should also cover any potential variation due to differing $\delta^{13}C_{methane.}$ Therefore the equation was calculated twice, once using $\delta^{13}C_{mob\_dip\ min}$ and once using $\delta^{13}C_{mob\_dip\ max.}$
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 $\delta^{13}C_{dip}$ and $\delta^{13}C_{hetero}$ (Table ~~1~~2). A threshold of 10% was used arbitrarily to identify the point at which we considered MOB to be contributing to the diploptene signal.

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 seeps m^{2} 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 (Figure 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.,

Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-17 10:45:03
based on marine studies??

Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 13:49:56
this section may become clearer with a re-organization on 1- CH4 signature for both lakes, 2- diploptene values for both lakes, and 3- mixed model results for both lakes. A paragraph on the relationship between diploptene (and/or mixed model results) and ebullition rate.

Nombre : 3 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 14:24:02
but above it is mentionned "and our own **summertime** bubble counts", they were both done? Clarify about all measurements (how, when, and how used for your correlation analyses; maybe in a table?)

Nombre : 4 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 13:28:03
m-2 d-1

Nombre : 5 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 13:27:49
rate?

Nombre : 6 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 13:28:53
zones

respectively. The $\delta^{13}C$ 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 ~2ka, indicating a dominant Holocene carbon source (likely decomposing near-surface peat). No radiocarbon date of methane were available at Ace L.

Diploptene was detected in all but one of the samples analysed (Table ~~2~~3; figure 3). This sample was not part of further analysis. The values ranged from -68.2 to -38.8‰ and had an overall standard deviation of 7.8‰.

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 (3.2m) and was the only sample that does not lie within 1 standard deviation of the mean for this thermokarst zone. However, another sample at the same depth was far less depleted (-50.1‰), which suggests the low $\delta^{13}C$ value is not explained by water depth. In Smith L., diploptene $\delta^{13}C$ 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-Whiney U test applied (H0: diploptene $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ values at Ace L.~~ake~~ were more negative than those at Smith ~~Lake~~ L. by at least 10‰, despite methane $\delta^{13}C$ values being less than 5‰ different. However, the samples in the thermokarst zone of Ace L. and the 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~~4. 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.

Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:27:56
is this the only age? It's rather thin, as readers expect that you will use this to support differences in diploptene among lakes or zones.

Nombre : 2 Auteur : Sujet : Barrer Date : 2015-12-18 13:31:20

Nombre : 3 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:28:41
this is an argument apparently in opposition to your above argument rejecting the deepest zone of Ace L. because it was too deep...

Nombre : 4 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:28:52
indicate that this comes from the mixed model calculation?

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 $\delta^{13}C$ values of diploptene ranged from -68.2 to -38.8 ‰ (Figure ~~3~~4), values similar to those that have been previously highlighted 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 $\delta^{13}C$ 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) systems, 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 some degree of MO was occurring, and the fraction of diploptene derived from MOB was >10% even under the most conservative assumptions (Table ~~3~~4).

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~~4). These estimates have a large degree of uncertainty ~~associated with them~~ and ~~W~~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.

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Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 14:07:00
signature?

Nombre : 3 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 14:04:46
results already given in result section

Nombre : 4 Auteur : Sujet : Barrer Date : 2015-12-18 14:08:49

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 and this methane might be derived from thermokarst specific sources.

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 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. However it could be argued that even if the methane that is being oxidised is from near-surface sediments, the high level of production is due to the lake type (yedoma) and the thawing and eroding margins. This might be a common pattern in these types of lakes and could be reflected in the $\delta^{13}C$ values of diploptene, however this needs to be tested with further research.

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 L. had diploptene $\delta^{13}C$ values that were similar to those of the Ace L. thermokarst zone. The differences between the centre and the thermokarst zone could arise from alterations in the

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why is this sentence placed alone? and I don't see that it's the case. To develop a model, you will need more than just 2 lakes, even if they would have provided the same trend (which is not even the case!).
The inference steps are diploptene to estimate MOB (biomass), to estimate MO rate, to link to dissolved CH4, to production rate, to emission at the surface (including ebullition)… This is a lot of steps. And the data base on which you rely to make all these links presents a large spatial variability, first in the diploptene signature, second in the link with ebullition rates (also considering your small representation of CH4 emission rates).
This study seems to have skipped scientific steps: first to establish the link between diploptene and MO rate with direct measurements of MO, second to establish the link between MO and CH4 production in thermokarst lakes (testing yedoma and non-yedoma sediment types)...

Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:31:46

I don't see any diffusion rate data to support this; you mean through a differing diploptene signature?
Yes this is logical (as explained in next sentence), but it is not apparently based on your data. I don't see anything in this paragraph that is linked to your own results (except that ebullition rate was high compared to Smith L.).

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 versus 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 ‘plastic’ 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, 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 near-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. Currently, as the data stand, it is difficult to decipher a clear pattern that can be linked to thermokarst specific methane production.~~, but~~

Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:32:01
why? Bastviken demonstrates that there is more MO in the center? please be more specific.

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what's that?

Nombre : 3 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 15:05:15
is this assumed or proven?

Nombre : 4 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:32:22
more than difficult, it is not possible to decipher any pattern

T~~t~~he results of this novel but preliminary study highlight the need to continue research in this area.

5.3 [1]Assessing past and current carbon cycling in thermokarst lakes

A crucial outcome of this study is the large variability seen in the $\delta^{13}C$ values of diploptene across small spatial distances which cannot be linked to specific types of methane production (e.g. near-surface or deeper, thermokarst specific ~~-~~production). This is an important finding, as often whole lakes can be represented by a single sampling site in palaeoenvironmental studies. Such large fluctuations in $\delta^{13}C$ 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 $\delta^{13}C$ values through time (i.e., down a single sediment core).

While the differences in diploptene $\delta^{13}C$ values between chosen study zones discussed above are statistically significant, [2]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 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 $\delta^{13}C$ values were used as a proxy for MO ~~that could~~to test whether these can be linked to variations in methane supply via diffusion in thermokarst lakes. Diploptene was present in almost all samples and its $\delta^{13}C$ 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. However the results were highly variable and suggest that, ~~L~~like methane production, MO is highly complex, both in terms of its spatial distribution and in relation to the type of

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is this the correct title for the content?

Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 15:12:24
It's especially the number of lakes (with both center and TK zones) that is small

Nombre : 3 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:33:10
this is unclear; when using higher trophic organisms remnants in core sediments in order to record past MOB thus past CH4 emissions??

Nombre : 4 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:33:45
you do not test that because you did not measure diffusion rates or dissolved CH4 in surface sediments where you collected diploptene; please be more specific on what you have done

substrate available. A single model for thermokarst lakes is unlikely to capture all patterns present at both the inter-lake and intra-lake level, and as the data stand, there is a large amount of variability which cannot be linked to specific types of methane production.. Thus, it is crucial that interpretation of diploptene $\delta^{13}C$ values (and other MO proxies) in palaeoenvironmental investigations take into account 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 given the current data, further research should be completed in order to understand the variability in d13c diploptene values prior to utilisation of this method for the reconstruction of methane cycling in lacustrine systems. 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.

Acknowledgements

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Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 15:21:23

this is unclear

Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 15:22:47

since you have used the work "patterns" for either ebullition vs diffusion or acetotrophy vs hydrogenotrophy, it is unclear what you are referring to here.

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Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-16 15:04:02

eliminate the first one and update all citation in the text body (including one that is missing the "et al.")

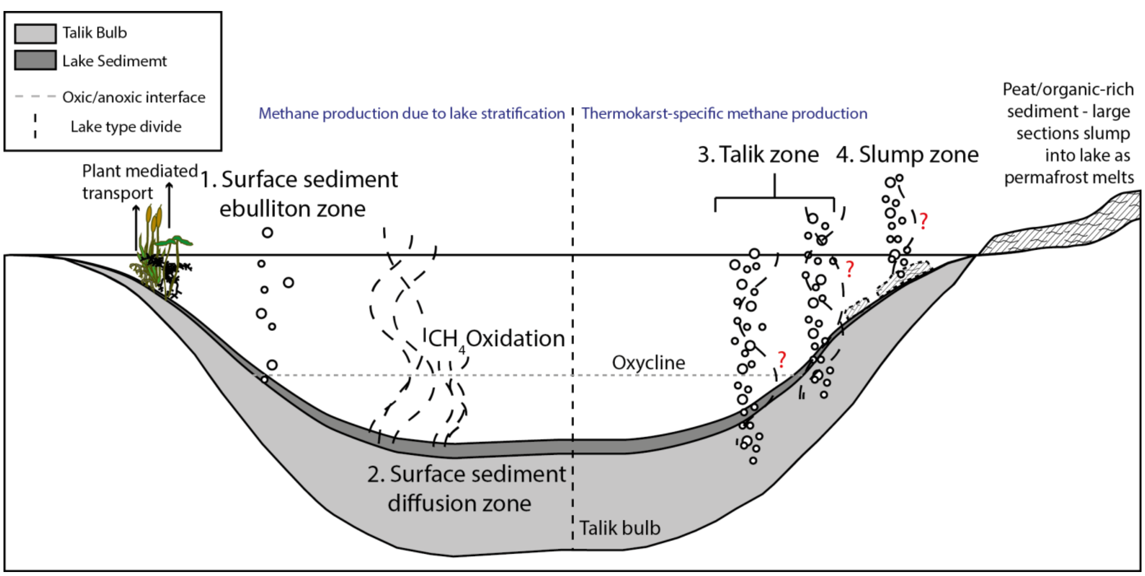
Table 1. Freeze dried sample weights for samples from Ace L. and Smith L.

	Sample No	Sample size (dry g)
Smith		
	1	0.2596
	2	0.2206
	3	0.3584
	4	0.1486
	5	0.1942
	6	0.5654
	7	0.3841
	8	0.2024
	9	0.3386
	10	0.2185
Ace		
	a1	1.3427
	a2	0.6812
	a3	0.5758
	a4	0.808

Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-18 15:31:22

I don't see the point in provinding these data and I am not sure this was asked by the reviewer

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.

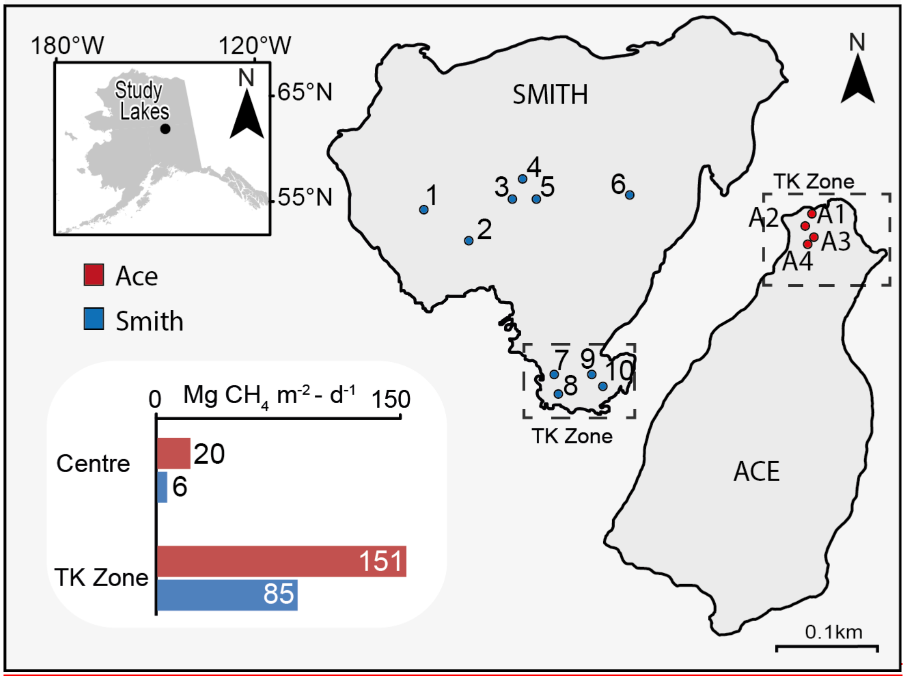


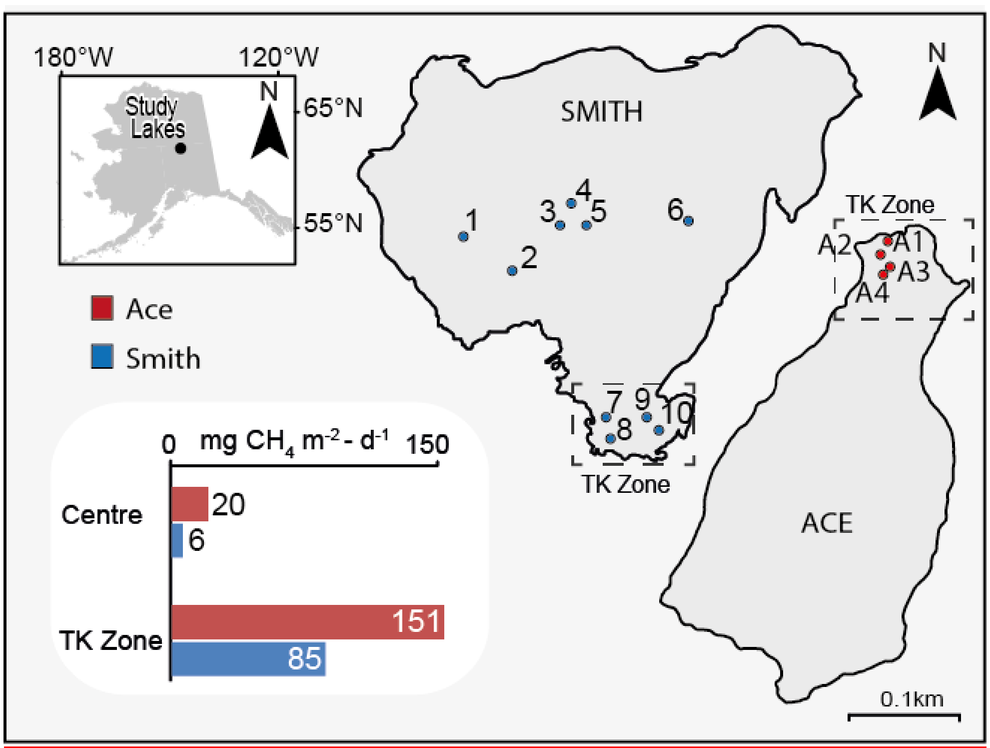
Nombre : 1 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-17 10:21:50
why? Is this background ebullition?

Nombre : 2 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-17 10:23:16
thawing

Nombre : 3 Auteur : Sujet : Commentaire sur le texte Date : 2015-12-21 10:34:47
replace by "questions marks"

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.





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don't you mean "averaged over the sites for each specific zones (TK or center)" ?
I suggest you repeat here the number of measurements used for these numbers, and provide over which time period they were taken at each sites.