

Answer to referee #1. “Recycling and fluxes...., by Nykänen et. al.). Referee comments received and published: 18 December 2014. Answers to referee are written in *italics* and improvements in corrected MS are **bolded**.

- 5 1. “I am sorry but this is a very weird manuscript. Not that I had only difficulties to understand what the purpose of this study is I also had to read sentences several times to understand what the authors wanted to say.”

10 *Referee #1 did not find much positive in our MS and the idea behind the MS was not clear to Referee #1. Furthermore there are many things referee #1 requests and argues to be relevant, which were not done during the study in 2007 - 2010, and cannot be done now. Therefore these issues cannot be addressed as the referee would wish, and the paper has to be evaluated based on the data that were collected. **We try to make the revised version clearer and better.** In any case Referee #1 stimulated a lively discussion about how to show something which does not exist.*

- 15 2. “A lake was fueled with cane sugar in two consecutive years, however, the comparison to the state of the lake before that addition is missing relevant data like CH<sub>4</sub> measurements.”

*As shown in MS, there were basically the same measurements of methane concentrations in year 2007 (before the cane sugar addition started) as in 2008 and 2009. Referee #1 probably means lacking methane isotope measurements in 2007. Unfortunately it was not possible for us to obtain isotope analyses of methane in 2007.*

- 20 3. “The data basis has much to low of a resolution to answer questions that are raised by the authors. We know that processes at the redoxcline are functioning on a millimeter scale (Kirf et al 2014, Aquatic Geochem.) but authors took samples at a 1 m, at best 0.5 m scale, this is not sufficient to address the raised question.”

25 *We think the referee is being unrealistic. We think that we can answer certain questions regarding processes in the water column with the resolution we now had. In general, the majority of articles concerning freshwater lakes report concentrations, physical variables and  $\delta^{13}\text{C}$  measurements with comparable resolution. If all such studies were only deemed acceptable if measurements were made with mm scale resolution, the aquatic literature would be almost empty. The resolution shown in the mentioned reference needs a totally different system and technical setting, which was not possible at this time and sampling*  
 30 *scheme we had. We agree that better resolution from critical depths would have been beneficial, and one set of measurements with 0.5 m resolution was obtained.*

- 35 4. “ I also question that in this lake anaerobic methane oxidation takes place (as quoted by the authors there is no isotopic signal suggesting this). It is most probably all aerobic oxidation since it is where oxygen and methane met where oxidation is seen (heavier isotopes in the remaining methane but this is very little as stated by the authors) (it is difficult to judge from figures 2 and 6 but resolution is too low). Calculation of fractionation based on this few data (resolution) is at best very coarse and it is very speculating to infer which kind of methanogenesis or oxidation took place.”

40 *The question about possible anaerobic/microaerophilic CH<sub>4</sub> oxidation was discussed in the MS (p. 16472, r 12 - 25). Also question if change in CH<sub>4</sub> isotopes is always a clear testimony of CH<sub>4</sub> oxidation was discussed. Methane isotopic enrichment was not detected in location where most of the oxidation took place according to diffusion gradient studies. But it was detected in oxycline, where concentration of CH<sub>4</sub> is low and then also quantitatively small oxidation causes big fractionation in residual methane  $\delta^{13}\text{C}$  values.*

45 *In general, this kind of stratified lakes are anoxic from their hypolimnetic water columns. And there is lot of evidence to still believe so also in this case as explained in MS (p. 16472, r. 20- 29). Possibility to microaerophilic CH<sub>4</sub> oxidation came to discussion mostly after article published in 2014 (Blees et al. 2014, LO, 59), due to this also this possibility is discussed (p. 16472, r. 12 - 20).*

50 *Even though we could not definitively prove absence of oxygen by our direct measurements, we also measured negative redox values, and found H<sub>2</sub>S from the water column.*  
***Furhermore, studies from the same lake have shown the existence of strictly anaerobic green sulphur bacteria in the lake; some at depth of 2.5 m, more at depth of 3.5 m and peak at depth of 4.5 m in summer 2009 (Karhunen et al. Aquatic Microbial Ecology 68: 267-272, Fig. 1). Also, Peura et al. (ISME J 6:1640-1652, 2012) found anaerobic bacterial communities from Alinen Mustajärvi. These findings will be added to MS.***  
 55

*Fractionation factors are not used here to evaluate where oxidation takes place, because they did not show that. Limit for aerobic and anaerobic oxidation is set to negative redox border.*

5. "2.2.1. Weather data is from a 18 km away, a long way, is this representative for the region?  
 60 What is the detection limit for the O<sub>2</sub> probe? This is absolutely essential to decipher oxygenated from anoxic layers. New sensors go down to nmolar concentrations, normal sensors are in the umolar range at best. Mentioned by authors that device does not give zero oxygen BUT this is ~essential here."

65 *This data from 18 km distance is well representative for the region and only possible showing long term trends in weather and general trends for the area in general. For flux calculation data from lake in 4 km distance was used.*

*As said in text, the detection limit was 0.3 mg O<sub>2</sub> L<sup>-1</sup>. Looking back, measurements using more sensitive probe would have been good, however those were not available.*

70 6. "2.2.5. were the samples treated with HCl to eliminate carbonate before 13C measurement?"

*POM and DOM samples were acid fumigated with HCL. Zooplankton, algae and biofilm was not acid fumigated. In general these small forest lakes in southern Finland (including Alinen Mustajärvi) are acidic and of very low alkalinity so that carbonates are negligible.*

75 7. "2.3.1 I do not understand how methane oxidation was determined. I do not see a concen- tration gradient really, also isotopes do not show oxidation. How were predicted (?) and observed

concentrations compared. Why weren't syringe incubations not done in 2008 and 2009 to compare them to values before carbon addition (2007)?"

80 *There is clear concentration gradient vertically (in bottom ~1000  $\mu\text{mol}$ , and in surface ~ 0.1 - 1  $\mu\text{mol}$ ) (Fig. 6 ACE, in MS). Calculation is based to turbulent diffusion with calculated theoretical values starting from measured bottom concentration value. Difference between measured and calculated values in watercolumn is amount of oxidized  $\text{CH}_4$ . This is repeated from time point to next measurement. Explanation of method is shown in Kankaala et al. 2006 (L&O 51:1195-1204). **This method will be explained more closely in corrected MS.** And as said in MS, "isotopes of  $\text{CH}_4$  did not show  $\text{CH}_4$  oxidation", and various reasons for that are explained (p. 16437, r. 1- 13).*

*Unfortunately, available time and resources framed our work, thus syringe incubations of methane oxidation were not done after 2007.*

90 **8. "2.3.2. What is an oxidation based estimate of production? The fractionation factors from Whiticar are now almost 30 years old and there are much more relevant fractionation factors in the literature which should be used. "**

*Oxidation based estimate of production is explained in reference (Bastviken et al. 2002, Env. Sci tech. 36). Basically, method is based on fact, that a measured amount of methane oxidized (+ that lost in ebullition, in outflow and in diffusion) must be compensated by similar amount of  $\text{CH}_4$  produced.*

95 *In general, Whiticar et al. 1986 equation (p. 16458, eq. 2) has been used a lot and is still used to evaluate methanogenic processes. When we have  $\delta^{13}\text{C}$  from  $\text{CO}_2$  and  $\text{CH}_4$  this offers a way to make an estimate based on two components taking part to methanogenesis in freshwater lake ecosystems. We did not have measured isotopes of possible other direct substrates, and generally acetogenic and hydrogenotrophic methanogenesis is regarded to be*  
 100 *far most important. **However, estimation of process pathway in  $\text{CH}_4$  formation is omitted in new version of MS, since possible methylotrophic methanogenesis having big fractionation will lead to wrong assessment towards hydrogenotrophic methanogenesis, as pointed out in reference shown by reviewer #2.***

105 **9. "2.3.3. This is not a method part but an introduction into how methane is formed. This should be clear to the reader. Why would sulfate be the oxidant? This is known from the marine environment. Is there any indication here?"**

**Since this part is too speculative for  $\text{CO}_2$  consumption estimates (p. 16458, r. 15 -25), it will be removed from the next version of MS. Also reviewer #2 doubted this.**

110 *Sulphate acting as oxidant is one possible candidate here, because  $\text{H}_2\text{S}$ , the product of oxidation with sulfate, was detected by its smell from this lake (p. 16472, r. 20 - 29). Schubert et al. Aquat. Sci. (2010) 72:455–466) found sulfate to be partly responsible of methane oxidation in Rotsee.*

115 **10.** “2.3.4. This whole description does not help the ms. since no values were measured but only some fractionation factors are used to estimate  $^{13}\text{C}$  of organic matter. Why haven't the authors filtered the water and measured the biomass directly? As it stands now it is a whole discussion based on some theoretical values. It is also not clear whether there is any anaerobic oxidation and hence biomass calculation rather questionable. The  $\text{O}_2$  detection limit of  $0.33\text{mg/L}$  is a huge amount”

120 *Fractionation factors are the only way to estimate methanotrophic microbial biomass  $\delta^{13}\text{C}$ . Luckily, our analysis of POM is just what referee asks (p. 16456, r.18). POM was extracted from 6 L of trough  $50\ \mu\text{M}$  sieve filtered water concentrated first by tangential flow filtration (pore size  $0.22\ \mu\text{m}$ ) to 0.5 L, which was immediately frozen and later freeze dried and analyzed with IRMS for C and N isotopes and used for DNA extraction (See Peura et al. 2014, Biogeochemistry 118, 177-194). Unfortunately separating methanotrophic microbial biomass to find out their isotopic composition is difficult because there is need to separate them from the other microbes, algae and other organic material, which is not possible.*

*Methanotrophs are only 3.7 % ( $\pm 3.5$ ) of total microbes (S. Peura personal communicaton). Separation may be possible from pure cultures in growing media, but not from humic lake water. One theoretical possibility, magnetic-bead-captured rRNA (Mag-Sip) (Miyatake et al. LO, 59, 2014) was not available to us. So, we had to use these values from literature in order to get at least some kind of estimate for methanotrophic microbial biomass  $\delta^{13}\text{C}$ .*

130 ***As referee 1 says, it may be too speculative and premature at this stage to guess what  $\delta^{13}\text{C}$  biomass from anaerobic microbial methane oxidation will be. This will be considered and may be removed and transferred to discussion part of article in some reduced form.***

*Oxygen detection limit is discussed above.*

140 **11.** “ The result section is a very detailed description of what is seen in the figure. It leads to no real conclusive results but is only a strung together of sentences. It is very hard to read/understand. What is the message? Could be at least cut by 50%.”

***This section will be rephrased and shortened and message will be shown clearly.***

145 **12.** “Again a separation of aerobic and anaerobic methane oxidation based on the shown data is not possible I have not seen any data that suggest methanogenesis in the water column, does this exist? References? Why use alpha and epsilon for fractionation, stick to one please There are very limited data on POM three depth once per year, an algae, some larvae, biomass floating around above the bottom. . .this is a very limited data base and now real interpretation can be done. Also the  $^{13}\text{C}$  values of those different species are then very different from what is estimated by using a fractionation factor and  $^{13}\text{C}$   $\text{CH}_4$  which questions the estimation very much.”

150 *We argue that separation of aerobic and anaerobic methane oxidation is possible based on measured redox depth and measured variables ensuring anoxia. Infact, our conservative choice of anaerobic layer depth (p. 16491, Fig. 5 b) is not based on oxygen measurements (P.*

16491, Fig. 5 a), thus we probably overestimate share of aerobic oxidation. We can also not show that there was microaerophilic conditions in water column. **However, this will be evaluated again and removed to discussion section if estimate looks more appropriate there.**

155

*Methanogenesis in anaerobic water column has been detected, to begin with Winfrey and Zeikus, 1979 (Appl Environ Microbiol. 1979; 37 (2):213-221.) and lately also in aerobic water column by Grossart et al. (PNAS 108, 2011 and Bogard et al. (Nature Communications 5, 2014) to mention few.*

160 **Fractionation symbol will be modified to be same in all cases.**

*POM data is average from 9 - 13 samplings annually as shown in reference to method (Peura et al. 2014, Biogeochemistry 118, 177-194), not once a year as referee claims. Due to limited resources only epi,-meta and hypolimnion samples were collected. In figure (p. 16492, Fig 6F) data from measurement with 1 m resolution in one case is shown, and it fits well to open water period measurements average.*

165

*Reviewer is right with scarce data of algae and zooplankton isotopes here. This may be too low amount of data to show how big share of methanotrophic biomass finally ends to diet of zooplankton. Zooplankton data collected with same resolution as other measurements here will be published later. However, Chaoborus was the only species living partly in the hypolimnion and thus can in theory use also biomass from bottom and can have the variable  $\delta^{13}C$  values detected. These values found don't question the estimate of biomass  $\delta^{13}C$ , since microbial biomass of methanotrophs having depleted  $\delta^{13}C$  values is only a fraction of their microbial (see above) diet and it also varies during the season. Thus big range found for  $\delta^{13}C$  (-37.9 - 24.5 per mill) don't turn down possibility to anaerobic  $CH_4$  oxidation with great fractionation of methane.*

175

180 **13."** Discussion Again here we find an ominous gathering of long interpretations which are not based on data. Whole paragraphs are copied from references and jumps back and forth from sugar addition to methane efflux to oxidation to biomass depletion are put together on a string. Sorry to say but this ms. is in my view only a first draft. The manuscript should be rewritten with a very clear focus and a red line to follow. Own data should be discussed in detail and not conclusions taken from other work and described in detail. I think there are some interesting data here, however, as it is presented now it is impossible to understand which point the authors want to make."

185 *Referee #1 claims that I (as a first author responsible of this) have copied whole paragraphs from references to discussion. This is a serious argument shown to all those reading open discussion. Fortunately I could not find whole paragraphs copied straight from the references nor did the program doing plagiarism checking.*

190 *I have to admit that my writing may not be enough clear and there may have been too many things combined to one manuscript, since these processes are interlinked and needed thus to be combined to one article. **In any case some of the more speculative parts will be removed***

*from the MS, and there are some improvements in clarity in later version, even getting MS to this stage was far from the first draft as reviewer claims. Our native English coauthor having some experience of writing and reviewing scientific articles as teaching scientific writing will be more strict with wording used in next version.*