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Interactive comment on "Methane production by three widespread marine phytoplankton species: release rates, precursor compounds, and relevance for the environment" *by* Thomas Klintzsch et al.

Thomas Klintzsch et al.

thomas.klintzsch@geow.uni-heidelberg.de

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Point-by-point response to the issues raised by referee #2 (Anonymous)

We thank the reviewer for efforts in reviewing our manuscript and for the helpful comments which have improved the manuscript.

Referee #2 The present paper presents an interesting study about methane production under oxic conditions in marine environments. This so called "methane paradox" is a very important research field to understand methane emissions from oceans (and

lakes) and has recently received strong interest by a number of investigators from different scientific disciplines. The author presents data from incubation experiments conducted with three different algal species. Methane production rates were determined with different methanogenic substrates (13C-labled) using a stable isotope approach. A similar kind of studies was previously conducted for Emiliania huxleyi by Lenhart et al. (2016) and the isotope approach was successfully used in diverse investigations by Frank Keppler before to examine terrestrial methane production. The novel outcome in the present study is (1) that also other widespread haptophytes have the potential to produce methane under anoxic conditions; and (2) methylated sulphur compounds (e.g. DMS), that are known to be enriched in the investigated algae species, present potential substrates. In addition, the authors present an attempt to transfer their results to an algal bloom in the Pacific Ocean to discuss the potential relevance of algal methane production. The experiments are well thought out and the results present an additional piece in the complex puzzle. There are lots of little corrections needed and from my point of view some sentences need another structure to make the content more accessible for readers that are not familiar with the topic in detail (especially in the method section, e.g. PP, exponential growth rate). I will give a few examples below. Some minor and major points need to be addressed and I therefor recommend a publication after major revision.

Authors: We thank the referee for the positive evaluation of our manuscript and for the helpful comments. Requested changes were taken into account, as detailed in the following.

Referee #2: Line 95ff. The experimental design is very complex. A flowchart for the method section would be helpful for the reader.

Authors: We added a graphic/flowchart to the method section of the revised manuscript.

Referee #2: Line 98ff. How clean are the algal culture samples (purity)? Small differ-

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ences in the degrees of contamination with archaea/bacteria (nitrogen limited bacteria, Line 69,Damm) between the cultures may have an impact on CH4 production rate. Does the web link give information about the purity of the culture?

Authors: Unfortunately, the weblink gives no detailed information about the purity of culture. We cannot consider our approach as axenic because it is extremely difficult to grow algal cultures without any bacteria. However, the algal cultures were diluted regularly, resulting in exponential algal growth and minimal bacterial growth. This is a common practice to keep non-axenic algae cultures largely free of bacteria (please see also answer to comment of reviewer #1 concerning manuscript line 98). We now mention that this cultures were non- axenic. We added the following sentence: "In order to keep non-axenic algae cultures largely free of bacteria, the cultures were diluted regularly, resulting in quasi constant exponential algal growth while minimizing bacterial cell density." However, we are aware that bacteria might play a role in CH4 production, but even if they did they still would depend on algal growth in our cultures as demonstrated by the following points.

1) The CH4 production rates decreased with decreasing algal growth rates: In batch cultures, the algae cultures undergo various stages of growth (see section 3.1, Fig. 1 a-c). Bacterial density increases tremendously when algae culture reach stationary growth phase and excretion of organic products from senescent alga cells together with the decomposition of cells is providing substratum for heterotrophs. This was described in literature (Salvesen et al., 2000) and is in line with our own experience with growing alga cultures in batch mode. In section 3.1 cultures have undergone transitionary growth phase leading up to the stationary phase. We calculated daily incremental CH4 production rates (not shown in the manuscript). The CH4 production rates of each species decreased with decreasing growth rates and decreased drastically when approaching stationary phase. This observation is the opposite of what we would have expected, if CH4 were mainly produced by bacteria. It would however be compatible with the idea that algae produce precursors which are subsequently used by bacteria

to produce CH_4.

2) Light is a prerequisite for CH_4 formation in algae cultures: Cultures of E. huxleyi, and P. globose were incubated under a day-night-cycle and continuous darkness. Methane concentrations did not increase when cultures were incubated in darkness while concentrations increased in cultures growing under a day-night-cycle. This is a strong indication that CH_4 formation is dependent on the light-dependent metabolism of the algae, since the metabolism of heterotrophs or archaea is independent of light. While the latter conclusion does not rule out the "algae precursor scenario", our experimental setup makes it rather unlikely. In these experiments we inoculated high cell densities ($\approx 10^5$ cells mL^{-1}) because they were designed to be short term which requires a high start cell density to yield measurable production. Therefore the start conditions will have included a seawater replete with precursors. It is unlikely that the relatively few bacteria present should have become precursor-limited over a single dark phase. It is rather more likely that the pool of precursors was sufficient to sustain bacterial CH_4 production over the dark phase. In this scenario an extra precursor production by cultures exposed to light would have been without effect on CH_4 production.

3) It is highly unlikely that methanogenic archaea are the source of CH_4 in cultures where CH_4 is produced alongside oxygen (incubation under day-night-cycle). If archaea were the CH_4 source we would have expected a higher CH_4 production in the dark.

4) Selectively inhibition of algal growth reduced CH_4 production rates: We compared emission rates of E. huxleyi that have been treated with and without 3-(3,4 dichlorophenyl)-1,1-dimethyl-urea (DCMU). DCMU acts as an inhibitor of photosynthesis (Wessels and Van Der Veen, 1956). Selectively inhibition of algal photosynthesis reduced both algal growth rate and CH_4 production rates. In the inhibition experiments, the growth rate was only 29% of the uninhibited culture and the CH_4 production rate dropped to 18% of the uninhibited culture. Since the inhibition effect of DCMU is very selective for algae (Francoeur et al., 2007) the result may indicate direct

CH4 production from algae. Although we regard it as unlikely, we cannot strictly rule out the "precursor-scenario": Bacteria use algae-derived precursors to produce CH4, and these bacteria require constant production of these precursors by algae. In other words, the precursor-production by algae is the rate limiting step of CH4 production by bacteria (as evident from points 1, 2, and 4 above). If true the CH4 production observed in our experiments would be the result of a "collaborative effort" which needs both partners, algae and bacteria. This would be a significant finding and prompt further research. Questions to be addressed would include: what are the precursors? Which algae can produce the precursors? Which bacteria can produce CH4 using these precursors? Is it possible to grow the respective algae without the bacteria (not all algal cultures can survive in an axenic state). Can the same CH4 production be achieved by growing the bacteria without algae and adding the precursors? This selection of questions would suffice for an entire research project. Meanwhile we are content with describing CH4 production that depends on algae, whether solely or in cooperation with bacteria. To sum up, our main finding is that CH4 production in mixed algae/bacteria cultures depends on algal growth and is not supported when algae become senescent. Future research will clarify whether algae alone produce CH4 or whether they produce precursors which in turn are used by bacteria to produce CH4. We have made this important point clear in the revised manuscript. We added this information in supplementary material. We now discuss the possible contribution of bacteria and archaea in the main text and refer to further discussion in the supplementary material (see discussion above). The revised the paragraph (Chapter 4.1) now reads:" The algal metabolites DMSP, DMS and DMSO are ubiquitous in marine surface layers and nanomolar concentrations were found in blooms of Chrysochromulina sp., P. globosa and E. huxleyi. Several field studies showed that these compounds are linked to CH4 formation in seawater (Damm et al., 2008; Zindler et al., 2013; Florez-Leiva et al., 2013). The authors proposed that DMSP and their degradation products DMSO and DMS are used by methylotrophic methanogenic archaea, inhabiting anoxic microsites, as substrates for methanogenesis. In addition it was reported that, if nitrogen is limited

but phosphorus is replete, marine bacteria might also use DMSP as a carbon source, thereby releasing CH4 (Damm et al., 2010). One scenario which we cannot rule out would be a production of CH4 precursors by algae and a usage of these precursors by bacteria to produce CH4. While we think that this is less likely than CH4 production by algae alone, it would, if true, show that bacteria need algae-produced precursors to produce CH4. The latter scenario would be relevant in the field because algae co-exist with bacteria in the oceans. Therefore bacteria might be involved in the CH4 production process in our cultures, but even if they were they still would depend on algal growth. For further discussion of a potential contribution of heterotrophs and/or methanogenic archaea see supplementary material. The correlations we describe in the supplementary material clearly show that CH4 production depends on algal growth. It is therefore highly unlikely that bacteria are solely responsible for CH4 production in our cultures."

Referee #2: Line 133. What is the difference in concentration of NaHCO3 between natural and inoculated water sample? Why did the authors added this amount of tracer? Should be mentioned.

Authors: Natural North Sea surface seawater contains ca 2000 μmol L-1 bicarbonate. We added 48,7 μmol L-1 13C-bicabonate , i.e. about 2 % of the natural concentration. This bicarbonate concentration was chosen for two reasons, one analytical, the other physiological. The physiological reason is that phytoplankton is sensitive to changes in seawater carbonate chemistry (see reviews on "ocean acidification"). We aimed at a negligible physiological effect of the added bicarbonate. The chosen bicarbonate concentration fulfills this criterion. The analytical reason is this: On the basis of the amount of added 13C-bicarbonate we calculated the theoretical δ13C -DIC value (see also manuscript line 134). Based on the theoretical δ13C -DIC value and from the previously determined CH4 increases in the cultures, the δ13CH4 values can be estimated. The amount of 13C- bicarbonate was chosen on the basis of expected changes of δ13CH4 values which were measured using GC-IRMS. A change of tenth to few hundred per mil is ideal regarding statistical issues (applying keeling plots for

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source identification) but also concerning linearity issues of the isotope ratio mass spectrometer.

Referee #2: Line 141. Here, you should explain in more detail why an exponential growth rate is important to best compare CH4 formation between the experiments. From this sentence one could assume that Langer performed already methane production rate experiments with algae that indicated that exponential growth rates are important. From my point of view the activity of the cell is important for the turnover of these substrates and not their reproduction. It should be mentioned in the method or result section that microbial methane turnover takes place in the incubations and the production rates presented are minimum rates > because methane oxidation is not considered in the calculations (e.g. see methods in de Angelis and Lee). Authors: We agree with the reviewer that from a physiological point of view the activity of the cell is the relevant parameter here. But as detailed below our point is purely methodological, not physiological. We have clarified this in the revised manuscript: "Exponential growth is a prerequisite for calculating production on the basis of growth rate and quota (here CH4 quota). The point is a general, technical one, and is not confined to CH4 production. The papers by Langer et al. (2012, 2013) discuss this point in the context of batch culture experiments. Briefly, production on this account is the product of growth rate and quota (e.g. CH4, calcite, organic carbon). Production here is an integrated value, typically over many cell divisions. For this calculation of production to be meaningful a constant growth rate is required. The exponential growth phase fulfills this criterion whereas the transition phase and the stationary phase do not. Therefore production cannot be calculated meaningfully in the non-exponential phases. The problem can, however, be minimized by using small increments (one day) because growth rate can be regarded as quasi-constant (see also Lenhart et al., 2016). "

We agree with the reviewer that our calculated rates should be regarded as minimum rates because of microbial methane turnover. Please see also answer to comment on manuscript line 98ff.

Referee #2: Line 137. Can you explain if aggregates or sediment was visible in the incubation?

Authors: Cells were counted under bright field microscopy and we did not observe any aggregates.

Referee #2: Line 172. Why was exactly this amount of substrate (DMS. . .) injected and is this comparable with natural environments (concentrations). Substrate concentrations definitely affect the turnover and the addition of tracers/substrates should not impact the sample too drastically. Why did the authors did not applied MET (and DMSP) as a precursor that was tested before successfully by Lenhart et al.?

Authors: The amount added was chosen based on the practical experience from previous experiments with E. huxleyi and methionine (Lenhart et al., 2016), so that the expected δ13CH4 fall within a measurable range with statistical significance. The growth of algae was not effected and changes of the overall CH4 production did not change by the addition of substrate (within error of measurement). The added amount (10μM) of methylated sulfur compounds (13C2-DMS, 13C2-DMSO or 13C-MSO) was higher than those expected in ocean water samples (please see also answer to reviewer #1 comment on manuscript line 133). However, the intracellular concentrations of these compounds can reach mM levels (Keller, 1989; Rafel et al.,1998; Keller et al., 1999; Sunda et al., 2002), which is two orders of magnitude higher than the added concentration of 10 μM (final) in our experiments. For this reason, it can be expected that the amount of 13C labelled substance taken up by the algal cells is low in relation to the amount of methylated sulfur compounds what they synthesize during metabolism. The turnover of DMS, DMSO or MSO (including non-labeled compounds) to CH4 could not be determined on the basis of their added amount of 13C-labelled substance in cultures of E. huxleyi. Please see answers regarding comments of reviewer#1 (manuscript 327), where we discuss this issue in detail. Neither was this the goal of the experiment. However, it can be determined exactly how the ratio of 13C in CH4 increases, when 13C labeled methylated sulfur compounds were added (Fig. 4 a-c). It has therefore

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been shown that methyl groups of these compounds can be converted into CH4 in algal cultures. Due to time constraints, we omitted the methionine treatment. Unfortunately, isotopically labelled 13C2 DMSP was not commercially available. Moreover, this compound could not be synthesized in our laboratories.

Referee #2: Line 327ff. Is it possible that a natural microbial community is needed for the turnover of these substances to methane? If the incubations are without contaminations (sterile filtered seawater, pure culture), the production rates might be low because of the missing community. The algae may only provide the precursors. Might be a point that could be discussed here.

Authors: Please see reply to referee #2: line 98ff .

Referee #2: Line 381. Argumentation is difficult. Only because Lenhart could prove a contamination-free incubations, this result cannot be transferred to all the incubation that will be performed by the working group afterwards. See comments/concerns to this topic above. Since the argumentation is difficult to follow, I suggest to discuss this topic less dominant and integrate this part somewhere else (not under a separate title). Also 50% of the text is nearly copied from the introduction (doubling!).

Authors: Agreed. This section has been modified according the referee's suggestions. Please see also reply to referee #2: line 98ff.

Referee #2: Line 335. I have a different impression. Figure 4a: At day 2 the d13C values are very close to each other. In Figure 4b all values from beginning to the end of the incubation time are very similar. Only Figure 4c shows a clear difference between culture and control over the course of the experiment. Add in the figure caption that also controls are plotted, not only results from cultures.

Authors: δ13CH4 values for 13C2-DMS are presented in Figure 4 a. We found that DMS is also converted chemically in sterile filtered seawater. This is in line with observations of Zhang et al. (2015) (please see manuscript line 332-339). However, the

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formation rates are very low and only become obvious when applying sensitive stable isotope labelling techniques. We agree with the reviewer that the δ13CH4 values of the DMS spiked seawater group and the DMS spiked algae group are very close to each other up to day 2. A section regarding control values was added to the revised manuscript (Chapter 4.1) :" The 13C2-DMS spiked seawater group and the 13C2-DMS spiked algae group are very close to each other up to day 2 (see Fig.3a and Fig.4a). For this time period, it can be assumed that the chemical conversion has taken place in both samples to the same extent, since the samples are relatively similar, because the algal cell density is only 5% (day 2) of the final cell density. However, the following days (day 3 to day 6), when algal cell density increased drastically, the δ13CH4 values of the algae cultures also increased significantly compared with δ13CH4 values of the seawater. This clearly indicates that conversion of 13C2-DMS to CH4 increases with increasing cell counts."

Referee #2: Chapter 4.3 I would recommend to perform an additional calculation to show that algal CH4 production is an important mechanism that can explain air/sea methane fluxes and methane enrichments. For example Schmale et al. (2018) gives detailed data about phytoplankton biomass (e.g. Prymnesiales) and production rates needed to maintain air/sea fluxes and subthermocline methane enrichments. There are probably also other papers available that could be used for such calculation.

Authors: We followed the recommendation and used the detailed data of Schmale et al. (2018) to estimate a possible contribution of algal CH4 production to the CH4 production rate in the field. An additional calculation was added to Chapter 4.3 in the revised manuscript. See reply to referee #1: line 400.

Minor issues:

Referee #2: Title: I recommend writing "potential relevance for the environment". A direct Transfer of laboratory studies/results into field observations is difficult.

Authors: Change applied.

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Referee #2: Line 24. Please also give the productions rates per cell in the abstract. Temperature is not needed to be mentioned in the abstract.

Authors: Changes applied.

Referee #2: Line 27ff. It should be mentioned here that the conversion of methylated sulphur compounds to methane was only responsible for less than 1% of the observed methane production (line 327ff).

Authors: We do not think that this information is important for the reader. The information might misleading here. Please see answers regarding comments of reviewer#1 (manuscript line 327), where we discuss this issue in detail.

Referee #2: Line 26-29. The word "clearly" is used to often.

Authors: We have rephrased line 26-29.

Referee #2: Line 30. "Relevance for the environment" is one major issue in the title but is reduced here to a little sentence. This part should be extended.

Authors: We have emphasized this issue in the abstract as requested. We added the following sentence: "By comparing the algae CH4 production rates with two field studies form the Pacific Ocean and the Baltic Sea we concluded that CH4 production could likely contributing to CH4 oversaturation in oxic waters."

Referee #2: Line 49. How can "emissions from freshwater" explain the CH4 concentration in ocean surface water?

Authors: We deleted "emissions from freshwater and" from line 49.

Referee #2: Line 50. Shorten the sentence and delete "that has been often: : :". "Well-known" means "often reported"

Authors: We have appropriately modified the sentence.

Referee #2: Line 55-58. This paragraph should be moved to line 46. It might be better

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to start with this overall review before listing the recent specific studies to oxic methane production in lakes and ocean.

Authors: As requested, we have restructured this section.

Referee #2: Line 60. May also mention Valle and Karl (2014) who used in situ MPn concentration in a 14C approach and showed that dissolved MPn in surface waters cannot account for methane oversaturation.

Authors: The revised manuscript contains the results of del Valle and Karl (2014) as well as the results of Repeta et al. (2016), who, on the other hand, reported that the cycling of the organic matter phosphonate inventory could be sufficient to support the total atmospheric CH4 flux at their study site.

Referee #2: Line 98. A bracket is missing (RC: : :). Is it clear for the reader for what the web link is good for?

Authors: The bracket was added. We have added the names of the culture collections to the front of the weblink to make it clear that the links lead to the respective collections.

Referee #2: Line 102. Delete “in” in front of “natural”

Authors: “in was” deleted.

Referee #2: Line 110ff. Why did the authors used different volumes (medium and headspace)?

Authors: For practical reasons. We have limited space in the climatic chamber and a limited supply of natural seawater, therefore the size of the vessels were adjusted.

Referee #2: Line 119. What is meant with “main cultures”? Is this the investigated culture in the incubation?

Authors: Yes, it means the culture that was studied during the incubation. We rephrased this sentence to make it clear.

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Referee #2: Line 119ff. I would suggest to transfer the cell densities to the result section (3.1).

Authors: Changes applied as suggested.

Referee #2: Line 122. E. huxleyi was sampled daily! What do you mean with overall sampling interval: 9,11,6 days? Why this odd order? And why did you sample the cultures in different intervals? If there is a reason for that it should be explained.

Authors: Overall sampling interval means the incubation time (from inoculation to the end of the experiment/incubation) for each species, that correspond to the sampling time. The incubation time varies from species to species and depends on the growth rate and the cell density in the stationary phase. The stationary phase for each species is dependent on a species specific cell density. As the species grow at different rates the sampling intervals differ. E. huxleyi has by far the highest growth rate and was sampled daily. Chrysochromulina sp. and P. globosa grow slower and were sampled at longer intervals. We revised this section in the manuscript and provided explanations.

Referee #2: Line 128. Why only three data points for E. huxleyi. From Figure 1c it seems to be plausible to use four.

Authors: The phase of exponential growth (from which μ was calculated) was defined by the cell densities which correspond to the best fit of the exponential regression. The fourth data point clearly deviates from exponential regression.

Referee #2: Line 131. It is always worth to have a repetition to support the previous results.

Authors: Due to the time constraints, we decided not repeat the experiments with stable isotope measurements that were already done by Lenhart et al. (2016).

Referee #2: Line 134. The delta is missing in d13C

Authors: Corrected.

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Referee #2: Line 135. Suggest to write: ": : :values of the methane precursor: : :"

Authors: The expression "source" is usually used in combination with Keeling plots. We would therefore like to keep the expression "source".

Referee #2: Line 144. ": : :measured at the end: : :"

Authors: Corrected.

Referee #2: Line 145. Suggest to write "For this additional experiment: : :"

Authors: Agreed. Changes applied.

Referee #2: Line 146. Suggest putting the cell densities in the result section (see above).

Authors: Changes applied as suggested.

Referee #2: Line 153. "ag" is the abbreviation for what?

Authors: Please see bracket in line 153. The unit "ag" means 10-18 g. The SI prefix "a" stand for atto (10-18).

Referee #2: Line 158. The program "Image J" is produced by which company?

Authors: "Image J" is an open source software. A reference was added.

Referee #2: Line 174. I still think that cell densities should be implemented in the result section (see above).

Authors: Changes applied as suggested.

Referee #2: Line 176. The target/design of the experiment in section 3.2 is still unknown!

Authors: We have revised section 3.2 to make the experimental target and design clearer. Please see also the answer to the comment concerning manuscript line 95ff.

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Referee #2: Line 189. Analyzed. See line 180

Authors: Corrected.

Referee #2: Line 193. Write: ": : : (based on three: : :)".

Authors: We changed line 193 as requested.

Referee #2: Line 204. Delete: ": : :at a temperature: : :". Here and in some other parts of the result section you mention details that were mentioned before in the method section. I would start with a sentence that makes clear that you are talking about the incubation with 13C-labelled hydrogen carbon (2.3).

Authors: Line 204 "at a temperature" was deleted. The result section was revised to avoid repetition of details from the method section. An introductory sentence was added in section 2.3 as suggested.

Referee #2: Line 208. Also here delete the repeated information: "These rates were obtained: : :". Check the entire result section to avoid redundancy.

Authors: The result section was revised to avoid repetition of details from the method section.

Referee #2: Line 211. The cell density should only be mentioned here and not in the method section!

Authors: All final cell densities were removed from the method section.

Referee #2: Line 214. Where is the control group plotted? Figure 2. Black and blue dots are difficult to distinguish. Even if it is "only" the control sample – make the visibility easier. The x-axis should be 1/CH4. Right?

Authors: Control groups in Figure 1 were plotted in the revised manuscript. Please also look at the answer to referee#1's comment regarding figure 1. Figure 2 was revised: by changing colors and correcting labeling of x-axis. Yes, "1/CH4" is correct.

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Referee #2: Line 249. See above. Not clear why the exponential growth phase is important and not the cell activity.

Authors: Please see the answer above to the comment concerning manuscript line 141.

Referee #2: Line 251. The equation is already described above (2 and 4). Avoid doubling. See comment above.

Authors: We revised this section in order to avoid doubling. The sentence "By doing so the CH4 production rate is the product of exponential growth rate μ and cellular or POC quota." was removed from line 251.

Referee #2: Line 254. The sentence should end with (Tab. 1).

Authors: Corrected.

Referee #2: Line 256. "community level" sounds odd in this context. May you can find a better description.

Authors: This term was used in the context of the production potential which was established by Gafar et al. (2018). The phrase "community level" is also used in this context by Gafa et al. (2018). We would therefore like to keep this phrase in order to avoid misunderstandings.

Referee #2: Line 266. It starts again with information that was mentioned before in the methods.

Authors: We removed this information from line 266.

Referee #2: Line 271. The sentence should end with "(Fig. 4)".Figure 4b. Change the x-axis to 13C2 (add 2).Figure 4c. Change the x-axis to MSO (not MES, see caption).

Authors: Line 271 and Figure 4c were corrected.

Referee #2: Line 279. Add the control sample in the Figure.

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Authors: Control groups were added.

Referee #2: Line 302. In the present study only the turnover of 13C-hydrogen carbonate by two algal species was investigate. Lenhart applied the isotope technique for E. huxleyi.

Authors: We have corrected this sentence.

Referee #2: Line 307. (with highest cell numbers) is out of context. Please rephrase.

Authors: The phrase "(with highest cell numbers)" was replaced by "(where the POC content is highest)".

Referee #2: Line 333. In future investigations I would suggest a dark incubation to exclude methane production by UV or visible light (line 70ff).

Authors: This topic is currently being investigated by us. Please see also answer to the comment of referee #2 concerning manuscript line 98. There we discuss a dark incubation experiment. In addition we discuss there a experiment with and without inhibition of algae by DCMU.

Referee #2: Line 352ff. Did Althoff really proved that the "reactivity" is the driving force in her experiments? Or are point 1 (label concentration) and 2 (penetration) also possible explanations for her observations?

Authors: Althoff et al. (2014) used a defined chemical system to study the conversion of methylated sulfur compounds to CH4. It turned out that the yield was not the same for all substances under otherwise identical reaction conditions. The CH4 yield was therefore also dependent on the substance and thus its reaction behavior.

Referee #2: Line 360ff. Sentence too complex. Devide in two parts.

Authors: As requested, we have reformulated and restructured this sentence.

Referee #2: Line 363 and 365. Too often "furthermore". Rephrase.

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Authors: Changes applied.

Referee #2: Line 391. Interesting. But it needs be explained in more detail why the growth rate impacts the methane production. See above.

Authors: A detailed explanation was added to the method section. Please see reply referee #2: line 141.

Referee #2: Line 411. Include/explain why PP is meaningful parameter.

Authors: Further explanation regarding the meaning of PP was added to the manuscript. Line 410 now reads: "Gafar et al. (2018) suggested the production potential (PP), as opposed to cellular production as a biogeochemically meaningful parameter because the PP includes the impact of growth rates on cell densities in an exponentially growing community whereas cellular production rates do not." âĂČ

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