

The authors wish to thank the referee for their efforts in reviewing our manuscript and for the helpful and constructive comments provided. Below are our point by point responses to all issues raised by the referee. The manuscript has been revised accordingly.

Anonymous Referee #1

General comments

Despite there are several suggestions to explain the methane (CH<sub>4</sub>) production pathway in the oxic ocean, the accumulation of oceanic CH<sub>4</sub> remains enigmatic. The idea that CH<sub>4</sub> might be produced by phytoplankton (algae) is not a new one; however, detailed studies on this issue are still lacking. Lenhart et al. present a novel data set of CH<sub>4</sub> production rates from a study with a *E hux* culture. The data and conclusions presented are of high interest for anyone dealing with the biogeochemistry of oceanic CH<sub>4</sub> cycle.

**Authors:** We thank the referee for their positive comment

However, and very unfortunate, the authors try to over-emphasize the significance of their results. The ms needs to be focused on the main conclusion (i.e. *E hux* has the potential to produce and release CH<sub>4</sub>). Any further far-reaching speculations about the CH<sub>4</sub> paradox are not justified by the results presented. Therefore, I can recommend publication of the ms only after major revisions. These are my points:

1) *E hux* plays an important role in the ocean, but of course it is only one of many algae species out there. Therefore the authors should avoid giving the impression that *E hux* is representative for all algae. Their phrases ‘. . . marine algae such as . . .’ (p.20325, l. 21) or ‘Since our results unambiguously show that algae are able to produce CH<sub>4</sub> per se under oxic conditions . . .’ (p.20344, l. 17/18) etc. have to be rephrased. This also applies to the title. To make it short, it is not acceptable to draw the conclusion that algae generally produce CH<sub>4</sub>.

**Authors:** As suggested by the referee we have made some modifications to the manuscript. We have modified phrase p.20325, l. 21 to read “Moreover, the absence of methanogenic archaea within the algal culture and the oxic conditions during CH<sub>4</sub> formation suggest that the widespread marine algae *Emiliana huxleyi* might contribute to the observed spatial and temporal restricted CH<sub>4</sub> oversaturation in ocean surface waters.” And phrase p.20344, l. 17/18 to “Since our results unambiguously show that the common coccolithophore *E. huxleyi* is able to produce CH<sub>4</sub> *per se* under oxic conditions we thus suggest that algae living in marine and freshwater environments might contribute to the regional and temporal oversaturation of surface waters.”

Furthermore we have revised the title to read “Evidence for methane production by the marine algae *Emiliana huxleyi*”.

2) Introduction: The oceanic source of CH<sub>4</sub> is negligible compared to other natural and anthropogenic sources of atm. CH<sub>4</sub> (see e.g. IPCC 2013). This is not mentioned in the introduction leaving the reader with the impression that the oceanic source is indeed significant for the global budget, which is not the case (see e.g. p.20326, l.25/26). Please modify the introduction and mention the oceanic source strength.

**Authors:** We have removed the sentence “In order to reliably apportion the global CH<sub>4</sub> budget, it is essential to know all significant sources and sinks and the principal parameters that control emissions.” from the manuscript and we now mention the ocean CH<sub>4</sub> source strength: “The world’s oceans are considered to be a minor source of CH<sub>4</sub> to the atmosphere with approximately 20 Tg CH<sub>4</sub> yr<sup>-1</sup> (Etiope, 2008).”

3) Introduction: The introduction needs a significant shortening and a focus of the main theme of the ms. There is a lot of information given which are not necessarily needed in the context of the ms. See e.g. paragraph about MPn as source of CH<sub>4</sub> (see p.20328, l.5-19) and other parts of the introduction.

**Authors:** We have modified parts of the introduction and removed a few sentences (e.g. comparison with freshwater ecosystems). However, we would like to mention the potential role of MPn for methane formation in the ocean (please refer to comment by referee 2 who asked to present alternative CH<sub>4</sub> formation pathways). Furthermore, we would also like to keep the introduction in its greater detail for readers which are not so familiar with biospheric methane formation under aerobic conditions and the CH<sub>4</sub> cycle in the ocean.

4) Please avoid comparison with freshwater lakes) and terrestrial (plants) systems which are not comparable with the oceanic ecosystems at all; there are several places in the text where a comparison with results from lakes and terrestrial plants are presented. Please modify.

**Authors:** In the revised manuscript we have omitted the comparison with freshwater ecosystems and have been also much more careful with the comparison of the emission rates with terrestrial plants.

5) I am wondering about different interpretation of the conclusions from Bange and Uher (2005). On the one hand, I read that photochemical production is ‘negligible under oxic conditions’ (p.20328, l.3). On the hand the authors cite Bange and Uher (2005) as being in line with their findings of a chemical CH<sub>4</sub> production found in their study which was conducted under oxic condition, I suppose. I think that this latter case is a misinterpretation of the results of Bange and Uher (2005). See also p.20342, l.25/26 where a photochemical CH<sub>4</sub> formation is listed as a potential CH<sub>4</sub> pathway in oxic surface waters. This is not correct; please modify.

**Authors:** We have modified the sentences taking into account the referees suggestions.

6) The section 5.4 ‘Methane paradox in oxic waters reconsidered’: This sections does not present any new results or conclusions and is way too much speculative. Therefore, it has to be omitted.

**Authors:** We have reworded the subtitle of this discussion section to read ‘Potential implications for the occurrence of CH<sub>4</sub> in oxic marine waters’. As this section belongs to the discussion of the manuscript we believe that it is helpful for the readers to discuss our findings in a broader context in relation to previous findings regarding precursor compounds and potential reaction pathways.

We would like to discuss the potential meaning of algae-derived CH<sub>4</sub> formation in oxic waters. However, we have reworded this section and changed the title of this section to “Potential implications for CH<sub>4</sub> cycling in oxic waters”.

Minor comments

Section 2.3 Gas Chromatography: Why are CO<sub>2</sub> and N<sub>2</sub>O mentioned? These measurements are not presented in the ms. Please correct.

**Authors:** Correction made

p.20334, l.5: ppbv is not a concentration it is a mixing ratio. Please correct. See also p.20334, l.15.

**Authors:** We changed concentration to mixing ratio throughout the manuscript.

p.20339, l.20: Schiebel et al. (2011) is missing in the ref list.

**Authors:** Correction made

p.20340, l.9: I could not find any information how CH<sub>4</sub> emissions (given in ng/gPOC/h) from the E hux culture have been converted to ng /gDW/h. Please explain.

**Authors:** The description is provided in the materials and methods section 3.4 “Calculation of CH<sub>4</sub> production” (20335 Line 7-16): “In order to compare CH<sub>4</sub> production to literature data it was necessary to normalize to cellular particulate organic carbon (POC) quota, as opposed to cell. The POC normalized CH<sub>4</sub> production is termed “methane emission rate” in the following. Since it was not possible to measure cellular POC quota on a daily basis, we used a literature value determined for the same strain under similar culture conditions, i.e. 10.67 pg POC cell<sup>-1</sup> (Langer et al., 2009). We are aware of the fact that cellular POC quota is likely to change alongside other element quotas when approaching stationary phase, but this change is well below an order of magnitude (Langer et al., 2013). For our purpose this method is therefore sufficiently accurate to determine POC normalized CH<sub>4</sub> production.

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**Referee #2:**

The manuscript by Lenhart et al. "Evidence for methane production by marine algae (*Emiliana huxleyi*) and its implication for the methane paradox in oxic waters" (bg-2015-628) reports on a highly interesting topic in aquatic biogeochemistry, namely the production and occurrence of methane in oxic water layers. Although it has been assumed that methane is rapidly consumed by methane oxidizers in the presence of oxygen recent studies have shown that methane in oxic waters is a common phenomenon, which is called the "methane paradox". However, sources and mechanisms leading to the accumulation of methane in oxic waters is largely unknown. Some studies have suggested a relationship between methane

concentration in oxic waters and primary production. Thus the study of Lenhart et al. provides a good basis for this assumption. The proof for production of methane by the common coccolithophore (*Emiliana huxleyi*) independent of the classical methanogenic (anaerobic) pathway indicates that methane can be produced by alternative pathways and following different dynamics in production and consumption than the classic methanogenesis. Therefore, I rate the manuscript of great interest for the readership of biogeosciences which has great implications for C-cycling and atmospheric gas-exchange.

The manuscript is well written and the results are stated in a clear manner. Consequently, I recommend the publication of the manuscript after minor revisions.

**Authors:** We thank the referee for their positive evaluation

I agree with reviewer #1 that the authors should not overemphasize their findings although they nicely demonstrate the existence of an alternative methane production pathway. Concerning the global relevance, however, the authors can only speculate since there are no direct measurements available. Therefore, I suggest to tone down a bit their general conclusions.

**Authors:** We have revised the manuscript according to the suggestions of referee# 1 and #2.

For example, the authors should still leave some space for alternative methane production pathways, e.g. via MPn and other methylated compounds which can so far not be excluded. In general, the real evidence of methane production directly by algae has to be still evaluated in field samples!

Introduction: Present alternative pathways, e.g. via MPn and other methylated compounds in a more neutral way since they still may significantly contributed to the methane accumulating in oxic waters.

**Authors:** We have removed the sentence "Thus, the environmental importance of this newly identified source remains open to critical debate." from the manuscript.

Material & Methods The methods are presented in a very structured manner. However, I am wondering that the algae were just kept at constant incubation conditions, i.e. 20C and 16:8 h light cycle. Please better justify why you have chosen for such conditions. In my opinion, to better estimate the global relevance of this process, you should incubate the algae under a variety of environmental conditions...

**Authors:** In this study the main aim was (as a proof of principle) to unambiguously provide evidence that *Emiliana huxleyi* are able to produce methane under aerobic conditions and without the help of microorganisms.

Results P20336: I suggest that the algae grown with <sup>13</sup>C labelled precursors grow less well than the once in the control because of lack in gas exchange. The authors may comment on this because it may differently affect the physiology of the algae.

**Authors:** We don't think that differences in gas exchange occurred, as all flasks were incubated under the same conditions.

The rest of the results is presented in a clear and defined manner.

**Authors:** Thanks very much

#### Discussion

P20339: The authors state: Contrary to the traditional assumption that *E. huxleyi* production in the Field is dominated by late summer bloom events, it was recently shown that non-bloom production in spring contributes significantly to yearly average production and therefore bloom events are not exceptionally important in biogeochemical terms (Schiebel et al., 2011). Therefore, I am very surprised that the authors did not test for other environmental conditions, e.g. at lower temperatures as can be found during the spring bloom and differences in light availability, nutrients etc. In my opinion, one cannot assume to always find the experimentally measured methane production rates... P20340: The comparison of the methane production rate should take differences in environmental parameters into account since temperature, light, nutrients etc. may be important factors determining methane production on land in a different manner than in water... At least I would mention this potential bias.

**Authors:** We agree with the referee that CH<sub>4</sub> emission rates will be influenced by environmental conditions. In our study the main aim was (as a proof of principle) to show that *Emiliana huxleyi* are able to produce methane under aerobic conditions and without the help of microorganisms.

The effect of environmental parameters such temperature, light and nutrient availability will be the focus of future experiments. We added a sentence about the role of environmental conditions that might control emission of methane from *Emiliana huxleyi*.

P20340-20341: The authors remain quite unspecific which potential process related to photosynthetic CO<sub>2</sub> fixation may result in the production of methane by the algae... May be they can add a bit more depth to this discussion.

**Authors:** The application of <sup>13</sup>CaCO<sub>3</sub> to the algae is an unspecific label and so far we don't know the specific pathway or the detailed mechanism that leads to the release of CH<sub>4</sub> from algae. The photosynthetically fixed CO<sub>2</sub> will be transferred into many metabolic pathways. We think that it's too early to speculate about potential processes of CH<sub>4</sub> formation from CO<sub>2</sub> or carbonate.

P20341: I wonder how bicarbonate uptake and methionine production are related to each other and how much of the methane production can be explained via methionine acting as a precursor. The importance of methionine as a precursor may again vary over time and may greatly depend on specific environmental conditions. At least it should be mentioned in the discussion.

**Authors:** We don't know how much methionine was synthesized from bicarbonate. As stated in the manuscript, about 3 % of the total amount of CH<sub>4</sub> produced by algae was derived from the <sup>13</sup>C labelled methionine added to the algae. We have added a sentence to the discussion that deals with potential precursors of CH<sub>4</sub> in *Emiliana huxleyi*. "Possibly, the formation of

potential precursors of CH<sub>4</sub> may change considerably under various climatic conditions, leading to varying CH<sub>4</sub> production rates in different pathways.” (20342 line 2).

In general, I miss a critical evaluation of the measured methane production rates. In my opinion the rates might be highly variable in space and time. In addition, the actual methane concentration in the water also depends on the methane oxidation. Hence the biogeochemical importance of the proposed methane formation pathway is very much dependent on a) the environmental conditions and b) on the balance between methane production and consumption.

**Authors:** We have added a sentence about the effect of environmental conditions to the manuscript (20340 line 10).

I suggest that the authors clearly state the need for future mainly field research to better evaluate the biogeochemical evidence of direct algal methane production.

**Authors:** We added a sentence about the importance of future field measurements that confirm direct formation from *Emiliana huxleyi*.