

## ***Interactive comment on “Evidence for methane production by marine algae (*Emiliana huxleyi*) and its implication for the methane paradox in oxic waters” by K. Lenhart et al.***

**K. Lenhart et al.**

katharina.lenhart@mpic.de

Received and published: 29 March 2016

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

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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,

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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 Emilia-

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*nia 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

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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*.

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Interactive comment on Biogeosciences Discuss., 12, 20323, 2015.

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