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Interactive comment on "Seasonal methane accumulation and release from a gas emission site in the central North Sea" *by* S. Mau et al.

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Interactive comment on "Seasonal methane accumulation and release from a gas emission site in the central North Sea" by S. Mau et al. Anonymous Referee #1 Received and published: 2 January 2015

Mau et al "Seasonal methane accumulation and release from a gas emission site in the central North Sea" (manuscript # bg-2014-506), provides a welcome analysis of the dynamics of methane flux in a seepage-influenced coastal shelf site. This analysis includes summer and winter measurements of methane concentrations and oxidation rates, in the context of thermal stratification and horizontal and vertical transport processes. The data and analysis contributes nicely to modeling the contribution of coastal marine sources to global methane inventories. General comments: The analysis was

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well-supported across various metrics. Incorporation of new data into the existing literature was also generally good, and the data fit well with past estimates of methane flux. The main conclusions are sound. I have several questions for the authors.

Specific comments: First, is it possible to determine if in situ methane production is occurring and biasing rate measurements? See e.g. Tang et al 2014, Limnology and Oceanography. This and other sources suggest that methane production in relatively shallow oxygenated waters can occur – possibly arising from methanogenic organisms tightly coupled to photosynthizers. Methane production and consumption may be likewise tightly coupled between microorganisms, and tracers may not compete well as a substrate in such a scenario (Furthermore – particularly when considering a low 'on rate'/'enzymatic uptake' as speculated in this paper, the added mass of heavy isotopes may introduce significant bias in rate estimates).

Author: In situ methane production is difficult to obtain due to the lack of knowledge, how methane is generated in oxic waters. One can determine the increase in methane concentration or use 14C-bicarbonate or 14C-acetate as it is done in sediments, but in the water column also dimethylsulfoniopropionate (DMSP) (Damm et al., 2010) or methylphosphonic acd (MPn) (Karl et al., 2008; Metcalf et al., 2012) have been reported as possible substrates. However, in the study area methane production is most likely much smaller than methane originating from bubble dissolution. For comparison, we measured a concentration of 20 nM (maximum was 25 nM) at a reference site, 32 km away, with an oxidation rate of 0.19 nM d-1. Even if one assumes methane production/consumption to be tightly coupled, these organisms appear not to be able to consume additional methane. Indeed, biological uptake tends to prefer light isotopes and a bias is most likely. However, the bias due to heavy isotopes is not known. Considering that a common isotopic effect is given in per mill and that duplicates differ by up to 30%, the isotopic bias might be arguable significant.

Second, the Michaelis Menten averaging may not be appropriate or valid. In Baani et al, two isoforms of pMMO are shown to have different kinetics of methane oxidation.

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On an environmental scale one expects widely disparate Km's from different isoforms, or homologs, of the same enzyme. Indeed, from a biochemical view, determination of Km is most appropriate from purified enzymes - and not necessarily reliably determined otherwise, yes? Is it possible to provide an indication of error in your averaged Km? Or, perhaps bin the pre-averaged Km measurements according to methane concentration to generate confidence levels that you are not averaging across different biochemical processes. There is more scatter in Figure 7 than I would have expected from the text.

Author: The range of possible Km is shown in Fig. 7A. These Km's are not related to the data. And yes, Km determined from an environmental sample is always a mixed Km of different enzymes and different quantities of different enzymes. We did not average any Km, we fitted a vmax and Km to our data (CH4-concentration and MOx) according to eq. 6 (excluding the 7 data points with MOx > 20 nM d-1). The fitted curve has a R2 of 0.81; we will add this value to the text. Using methane concentrations from 1 to 500 nM and calculating the MOx taking eq.6 with the fitted parameters vmax and Km, we can calculate k', which is MOx divided by [CH4]. Therefore, we use the equation and fitted parameters to derive a k', which is based on 120 data points. If this method is used with other data of other regions, it might provide an understanding of the difference of vmax and Km in environmental samples. The scatter in the figure appears more significant than described in the text, because a lot of low rate and concentration measurements plot onto each other while higher rates and concentrations do not, but have been less often measured.

Lastly, it is not clear if you are posing that there is, or isn't, a microbial methane oxidizing community. What seems likely is that microbial methanotrophs are present in such low numbers and/or are such poor matches to your PCR primers that they are below detection. The latter of these possibilities has been directly demonstrated for marine planktonic methanotrophs (Hansman, 2008). Additional research using not only PCR but also methods with intrinsically less bias (e.g. SIP, metatranscriptomics) has demonstrated global cosmopolitan presence of canonical as well as unusual methanotrophs

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in bottom waters. These published findings pertain directly to the problem of primer bias in PCR (e.g. Li et al 2014, Env. Micr.) Shallower marine waters (<_200m) in the Pacific, Atlantic, and the Gulf of Mexico are almost invariably devoid of detectable canonical methanotrophs - but can host unusual pmo variants including those from unusual phylogenies. Your results from a relatively shallow marine source relate directly to these published trends (e.g. Tavormina 2013 and references therein).

Author: We appreciate this constructive comment and will add a few sentences stating your suggested possibility that known methanotrophs were not detected due to low number or inappropriate PCR primers. By using the common primer sets for the pmoA gene analysis, we did not obtain a positive PCR product, but we know that this does not mean that methanotrophs were absent. Other methods might be able to discover methanotrophs that did not match the used PCR-primers. We also will state that our finding is in accordance to others in shallow marine waters.

Technical comments:

Twice the word 'ascend' should more properly be 'ascent,' on page 18006 line 9 and page 18019 line 12 (Please also remove the comma following 'vent sites' on line 12). Page 18009 line 16: The word 'gaschromatograph' is two words in the English language. Page 18018, line 13: Please change 'but showed also' to 'but also showed' to correct the grammar.

Author: All these suggested corrections will be done.

Can you provide some interpretation of these results? Do you believe that so few bands were clearly resolved because the original product was phylogenetically diverse, or was the quality of the original product poor? I would be curious to see the non-denaturing gel, to have a sense of the efficiency of the initial amplification reaction.

Author: We think that our DGGE-results are reliable, because we filtered 8 l of water, had good DNA-extracts, and used sufficient DNA for PCR. The quality of the winter

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samples was not as good as the quality of the summer samples, but from our experience with pelagic samples, the results are plausible, too.

Page 18019 line 25 through page 18020 line 5: It may be appropriate to add Narvenkar et al 2013 to these references.

Author: The reference will be included.

Page 18027 line 20: The Kessler estimates, which are environmentally based from a marine system, are more relevant than estimates from terrestrial organisms grown in culture. It may be appropriate to mention that few if any planktonic marine methanotrophs are currently available in culture thus doubling times are challenging to estimate.

Author: The suggested statement will be included.

Table 2. Crespo-Medina (Nature Geoscience 2014) recently reported Mox rates surpassing those included in this table. Consider inclusion. Author: The reference will be included to complement the table.

Interactive comment on Biogeosciences Discuss., 11, 18003, 2014.