Response to reviewer comments.

We would like to thank all reviewers for their effort to provide a constructive review and suggestions to improve the manuscript. We considered each comment and included most of the suggested changes. In those cases where changes were not made, we explained why we disagree or the changes are not possible to add. Our replies can be found below each comment.

For an overview; the main changes comprise:

- Added a short, general description of oceanographic aspects relevant for the study
- Added estimation of advective transport
- Added more information for sea-air flux estimation
- Added description of current speed during sampling campaigns using modelled forecasts, the data is shown in Supplementary Material 2 and 3
- Added data of reference station in Supplementary Material 4
- Revised the averaging of the constant *k'* and added a discussion why no methanotrophs were detected with the molecular methods used
- Estimated critical parameter D_V of model according to the equation by Osborn (1980) relating the dissipation rate of turbulent kinetic energy with the buoyancy frequency
- Added a sensitivity test of the model to the discussion of model results
- Added comments why methanotrophy remained low even methane was sufficiently available
- Changed some references

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 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 ^{14}C-bicarbonate or ^{14}C-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. 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 K_m is shown in Fig. 7A. These K_m's are not related to the data. And yes, K_m determined from an environmental sample is always a mixed K_m of different enzymes and different quantities of different enzymes. We did not average any K_m, we fitted a v_{max} and K_m to our data (CH_4-concentration and MOx) according to eq. 6 (excluding the 7 data points with MOx > 20 nM d^{-1}). The fitted curve has a R^2 of 0.81; we added this value to the text. Using methane concentrations from 1 to 500 nM and calculating the MOx taking eq.6 with the fitted parameters v_{max} and K_m, we can calculate *k'*, which is MOx divided by [CH_4]. 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 v_{max} and K_m in environmental samples. Please note, that we revised this statement according to our response no. 5 to reviewer 3 (Page 15, Line 518-519).
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 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 added 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 stated that our finding is in accordance to others in shallow marine waters (Page 16, Line 534-540).

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 are 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 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 6, Line 209).

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 is 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 methananotrophs are currently available in culture thus doubling times are challenging to estimate.

Author: The suggested statement is included (Page 20, Line 686-687).

Table 2. Crespo-Medina (Nature Geoscience 2014) recently reported Mox rates surpassing those included in this table. Consider inclusion.

Author: The reference is included to complement the table.

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 #2

Received and published: 11 January 2015

the paper aims to constrain the seasonal variation of sea-air methane fluxes originating from shallow gas seepage, which is an important research question. The area of interest is located in a summerly thermally stratified part of the North Sea showing complete mixing in winter. A two layer model is introduced to investigate the seasonal changes of physical methane fluxes and relate those to microbial uptake.

The paper tackles complex tracer oceanographic problems requiring well planned sampling strategies, current measurements, solution of advection-diffusion equations and estimates about seasonal variation of vertical eddy diffusive transport. But the paper only presents an extremely simplified model. The oceanographic understanding appears limited and the model suffers from incompleteness and severe misunderstanding.

Author: The model is only part of the reported work and thought to support our conclusions, which were based on our data. It is not indented to be and nowhere near a detailed oceanographic model, which would need a more comprehensive data base. Our simple model is rather thought to check if our hypothesis to explain our data is correct. Biogeochemical measurements in summer and winter time in a shallow shelf sea area

indicate that a seasonal thermocline leads to an enrichment of methane below the thermocline. The winter data, which is currently rare and worthwhile to be published, shows that without a thermocline, there is no enrichment of methane. Furthermore, we discuss that microbial methane oxidation appears to be a small sink of methane. These findings hints to the conclusion, that most of the methane, which piles up below the seasonal thermocline, must be vented to the atmosphere as soon as the thermocline breaks down. We used the model to check if that hypothesis is plausible. Certainly more data collected at different times of the year and revised models are needed to further validate this hypothesis.

(1) The vertical eddy diffusivity kz was estimated constant to 10e-4 from literature (and tested for model uncertainty with 10-3, and 10-5 respectively. A seasonal built-up and destruction of a thermocline gives rise to a non-static kz with variation by orders of magnitude throughout the year. The authors should have derived monthly kz, e.g. by Thorpe Scale analyses, from CTD data.

Author: First of all, we used only one value of k_z (D_v in the text) for cross pycnocline mixing in the months from May until August, when there are two different water layers. The rest of the year, the water column is well mixed and there is only one water layer.
Unfortunately, we do not have sufficient CTD data to be able to calculate representative k_zs. The data for the months May and June originate form an area within 3 to 6° E and 54 to 56° N as described in the manuscript, but were located closer to the coast than our study area. We lack any CTD-data of August. All CTD-data originate from water sampling and were not taken to investigate the turbulent diffusivity, thus we do not have sufficient replicates for a representative estimation of k_z. However, based on the constructive suggestion of the reviewer, we estimated k_z by using published dissipation rates of turbulent kinetic energy (Palmer et al., 2008, Thorpe et al., 2008) and calculating the buoyancy frequency from the available CTD-profiles. These results indicate that k_z is in the order of 10^{-4} to 10^{-6} m^2 s^{-1}. Therefore, the value of k_z used for the model was appropriately chosen. We included this estimation in the manuscript (Page 18-19, Line 626-637).

(2) A 1D model is suggested to describe the flux of methane from the “deep” layer to the upper/mixed layer using Ficks 1st law. The authors derive model parameter dC_CH4/dz from their field data by assuming a 1D case. This would require a distinct dC_CH4/dz gradient with more or less homogeneous horizontal distribution of methane. However, the near field water column methane distribution pattern surrounding individual gas seepage clusters appears highly variable, i.e. with significant variation in three dimensions as shown by the authors themselves (Fig. 4). Surface methane values measured up to 2127nM with UWMS were reported. Obvious reasons are gas bubbles as visualized with acoustics. But the model assumes the only CH4 source is the lowermost layer in their model. In summer the thermocline may reach down by 30m leaving a lowermost layer with 10m thickness. Methane gas bubbles easily bypass a 10-20m bottom layer without losing major fractions of their initial moles as shown in the cited paper McGinnis et al. (2006). No field data is provided about the crucial model parameters initial gas bubble size and methane mole fraction. Overall, bubbles most likely provide a strong source for methane input to the upper layer, but this is totally neglected in the model.

Author: 1D models are also used for modeling transport and reactions in marine sediments where methane and other chemical compounds also considerably vary over space. Similarly, bubble dissolution models do not take horizontal advection into account. These models (Leifer and Patro, 2002, McGinnis et al., 2006) suggest highest dissolution close to the sediment surface with an exponential decline towards shallower depth, thus, the main methane contribution occurs in the bottom layer, which is coherent with our data. Indeed part of the bubble dissolution takes place also in the upper layer, but if we assume that bubble dissolution is not affected by stratification as shown by Schneider von Deimling et al. (2011) at the Tommeliten site in the North Sea, then the source term for the upper layer would

always be the same. Therefore, the model values that are presented are conservative estimates and the model results are qualitatively correct.
Gas bubble size and methane mole fraction were not measured during the two field campaigns as our intention was to investigate the seasonal variable methane distribution.

(3) monthly mean wind speed was taken for sea-air flux modeling. But the sea-air gas transfer is highly non-linear with wind speed and a monthly mean approach needs discussion. The sea-air flux potential is also governed by an interplay between strength and continuation of wind in relation to the remaining dissolved methane pool in the "wind-exhausted" layer. I.e. strong wind will not necessarily drive enhanced sea-air flux once the upper CH4 layer was exhausted already.

Author: The formation and erosion of the seasonal thermocline forming a kind of barrier for dissolved methane was the objective of the model, thus, we used months as appropriate time period. Furthermore, monthly values of wind speed were readily accessible. However, based on this comment, we conducted a sensitivity check varying the parameters by +/- 10%. The sensitivity analysis showed that wind speed is the most sensitive parameter inducing a change of ~25% whereas surface water temperatures only yielded a difference by 1.5%. We added the sensitivity check to the discussion (Page 19, Line 659-663).

(4) The box model approach is only feasible in a closed system, but most likely the sampled area is an open system with significant advection and methane loss in various directions, and gas bubble methane dissolution up to the sea surface. The paper refers to using the disputable approach from Mau et al. (2012).

Author: We used a 1D model that indeed ignores any horizontal movement of methane. This very simple approach was used to test our understanding of the system. If horizontal effects would be taken into account, then the source term would need to be increased. The added quantity of methane would be horizontally advected and dispersed, but the vertical exchange processes would remain the same, thus, the outcome of the model is qualitatively correct. If we move horizontally away from our modelled source, then all concentrations would be lower due to horizontal eddy diffusion. Therefore, the limitation due to the thermocline would diminish with distance to the source and the sea-air flux would decrease. As we did not extrapolate over an area, but focused on the emission site, we argue that the model is sufficient for identifying a hypothetical seasonal cycle. More importantly, as the results fit to our observations, we think that the model is a valid approach.

(5) No current data are presented for the study site. But the North Sea is highly affected by the tides and the dominating M2 tide will likely cause significant current changes in amplitude and phase on an hourly timescale. Therefore the tempo-spatial methane distribution and the respective sampling are highly controlled by the actual current around the seep sites. The methane distribution pattern in winter shown in Fig. 4b is interpreted as a result from enhanced mixing. However, it could also be caused from current amplitude and direction "flushing" the seepage area with background water during the time of measurement (e.g. frontal jets have been discussed for the Dogger Bank with currents exceeding 15 cm s -1, but are not mentioned in the paper).

Author: We agree that currents affect methane distribution, however, currents transport the methane, but do not decrease the concentration as no concentration gradient is included in the equation of the advective flux. We drew a sketch to show the influence of advection/currents (Fig. 1) showing that independent of the current velocity, the vertical concentration profile would always look similar: the concentration would exponentially decrease with distance to the seafloor. In addition, the seasonal thermocline will always limit vertical transport, thus, the model is qualitatively correct, but not necessarily quantitatively.

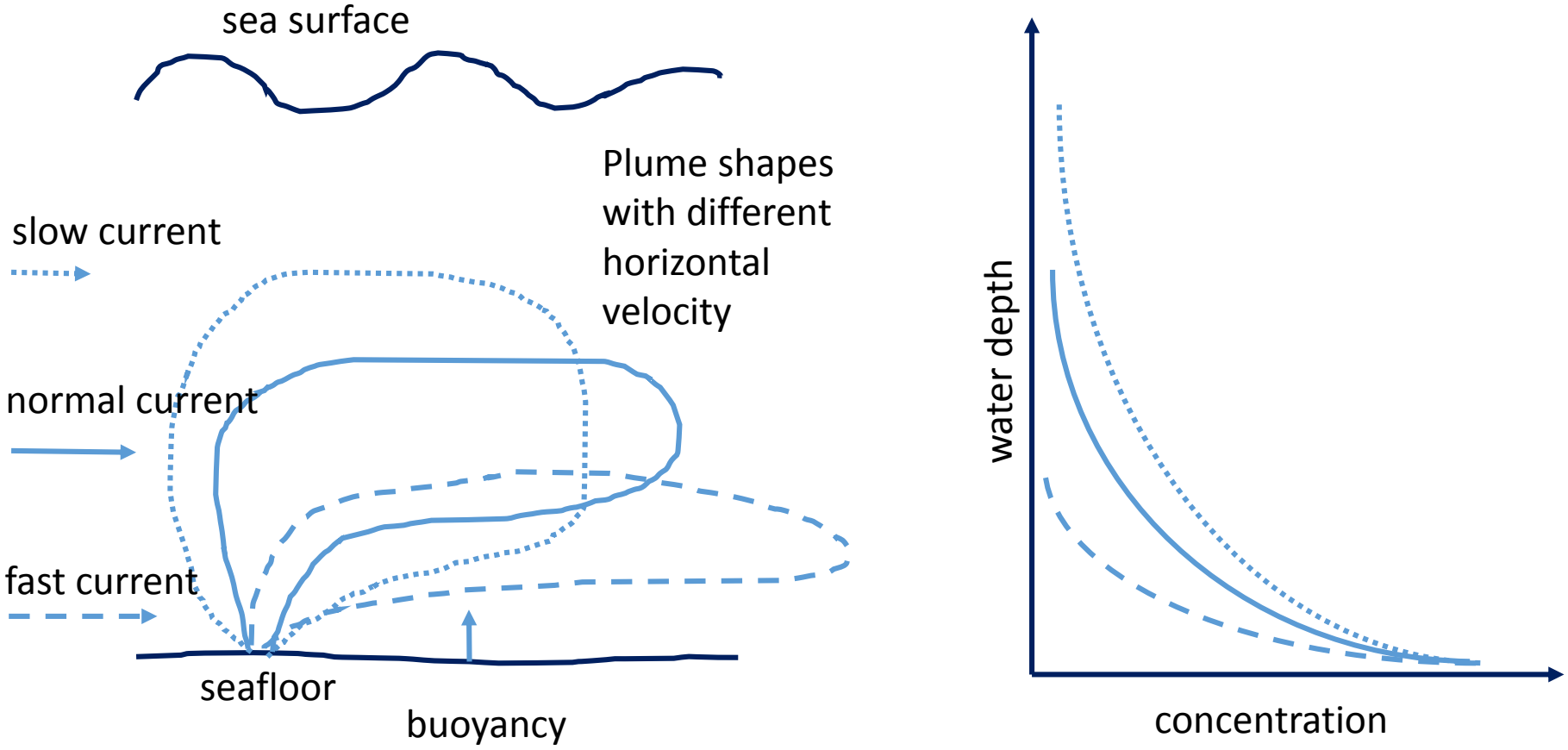

Fig. 1: Sketch of a methane plume with a constant source and buoyancy, but different water velocities. The shape of the vertical methane concentration profile would not change.

However, we included a description and added a plot to the supplementary material showing the east-west and north-south velocities of the currents during the sampling campaigns (Page 10-11, Line 348-355). The current data are modelled data by the BSH using wind and air temperature forecasts, thus, might be deficient. These data indicate that current direction was for most CTD stations to the north-west. Also the velocities were similar ranging between 0.1 and 0.2 m s^{-1} during sampling campaigns. The lower concentrations in winter (i.e., western part of transect) were measured when the currents were less intense than the currents during sampling the eastern part of the transect, where higher concentrations were measured. Therefore, there was most likely no flushing.

No background CTD is available, and the amount of lateral input of methane into the layers remains unknown.

Author: Methane concentrations measured at a reference station were mentioned and we provide the data of the background CTD sampled 32 km away from the central station in summer (Page 11, Line 368-369). Methane concentrations at the reference station ranged between 17-25 nM and agree well with the ones published by Grundwald et al. (2009) for the German Bight.

The observation of enhanced MOx activity at depth is a valid observation. Also the high resolution in situ mass spectrometer CH4 data in the near-field of gas seepage is valuable, because such data are very rare (but the respective 3D methane distribution it is not presented in the paper). The authors could think about a complete new story using such data. With the severe shortcomings of the model and missing current information the content of the paper can not support the conclusions. Therefore this paper can not be suggested for publication.

Author: We think that our conclusions are justified. Our main conclusion is that dissolved methane accumulates below the seasonal thermocline in summer and does not accumulate in winter when there is no stratification. Furthermore, detailed analysis of the methane oxidation measurements illustrate that methane oxidation is insufficient to significantly reduce the methane load. In combination, this suggests that the accumulated methane is at some time transferred to the atmosphere. Therefore, the conclusions are drawn from our measured data and, in addition, are supported by the model, which we argue to be qualitatively valid. Our conclusions and measurements fit to and extend the current knowledge of the sinks of methane emitted from shallow seep sites. We provide analysis in winter time and methane oxidation measurements, which are both rare data and reinforce the hypothesis of a higher methane release from the sea to the atmosphere in fall as reported by Gülzow et al., 2013 for the Baltic Sea.

Technical Comments - equations are missing to allow for reconstructing the individual model steps

Author: On page 18014 line 12 we describe that eq. 3 and 4 are used in the model.

- Fig. 1: the wind recording station can hardly be detected. The flow pattern of the North Sea currents are provided, but the ones prevailing in the study area remain unclear, also in the text!

Author: We changed the color of the wind recording station for better visibility and included a description of the general oceanography and available modelled currents during the samplings campaigns (Page 4, Line 106-115; Page 10-11, Line 348-355).

- Fig. 2: poor quality and unclear. The three figures/inserts have three different color codes for the depth, confusing: : :.The UWMS sampling path could be better presented in 3D together with the methane concentration distribution in the results chapter.

Author: We changed the color code of the UWMS sampling path matching it to the flare imaging color scale, the other depth scale refers to the smooth topography. If we would have a scale for all, then the topography cannot be illustrated (would be one color).
We think the discrete measured water samples and the UWMS-data are best compared as we illustrated it.

- Fig. 3: The CH4 concentration profiles should be included here.

Author: The different methane concentration profiles are not as similar as e.g. the different temperature or salinity profiles, thus, we presented the data as contour plots to show more details.

- Sea-air flux calculations: rather provide classical and comprehensive work introducing the generic sea-air flux equation equ. 4 than self-citation.

Author: The citations were deleted.

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 #3
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The manuscript of Mau and co-authors describes the distribution of methane at a methane seep in the North Sea, together with the microbiological methane consuming process, its oxidation. The authors provide data from classical water sampling and additional in situ measurements of the methane concentration. Methane oxidation rates were measured with 3H-tracer, as an appropriate method. Thus, altogether a valuable data set. However, I have difficulties with the modelling part and the kinetics of the Mox rates of the manuscript. I therefore suggest "major revision“.

Modelling 1) Based on the in situ data of methane, the authors have a 3-dimensional data set at hand. Thus it is not clear, why they restrict these data to simple box plots and further restricts the modelling to a 2-dimensional model. At the study site a 3-dimensional model with transport processes in all (or at least 4) directions would be more appropriate.

Author reply 1: We show 2D methane concentration data that are concentration profiles throughout the water column taken along a 6 km transect. We also show in situ UWMS-measurements that were collected near an active seep site in a very small area of 125 m by 150 m (Fig. 2). Therefore, the transect data show a much larger scale than the UWMS-data. The UWMS-data were included to prove that methane concentrations are similarly distributed over the tidal cycle in summer. For comparison of the two data sets (discrete sampling by CTD/rosette and UWMS-records), we found that a contour plot of the discrete samples and a box plot of the UWMS-records are most illustrative.
The model was used to correlate summer and winter methane concentrations and methane oxidation rates collected along the transect. The model is restricted to 1 dimension, the vertical dimension or height of the water column, which is (1) defined by the direction of the sea-air flux, which is one of the major sinks of dissolved methane in the water column. Furthermore (2), the main difference between

summer and winter data was the seasonal thermocline, which limits the vertical transport also in the dimension of the water column height. As we mentioned in the response to the second reviewer, if horizontal effects would be taken into account (3D), then the source term would need to be increased. The added quantity of methane would be horizontally advected and dispersed, but the vertical exchange processes would remain the same. Moreover, we would have been forced to include additional parameters such as the horizontal eddy diffusivity that all add uncertainty to the outcome of the model.

2) The transport processes used for the modelling are not clear to me. The authors neglect the advective process, as " currents only transport the water from the methane seep away". However, by doing so, at the study site the methane concentrations will decrease as methane rich water from the seep will be displaced / mixed with methane poor water. Thus, I think that dilution / mixing of water bodies through currents is an important factor, which should not be neglected.

Author reply 2: The same issue was raised by the second reviewer. We include our response to the other reviewer here again: We agree that currents affect methane distribution, however, currents transport the methane, but do not decrease the concentration as no concentration gradient is included in the equation of the advective flux. Largier (2003) put it that way: "The diffusive flux is then determined by the ''eddy diffusivity'' *K* that parameterizes the strength of small-scale motions and acts to smooth out gradients (whereas advection simply displaces them)." and also "advection is the mean movement and diffusion is the spreading out." We drew a sketch to show the influence of advection/currents (Fig. 1 in the response to reviewer 2) showing that independent of the current velocity, the vertical concentration profile would always look similar: the concentration would exponentially decrease with distance to the seafloor. In addition, the seasonal thermocline will always limit vertical transport, thus, the model is qualitatively correct, but not necessarily quantitatively.

3) The description of the turbulent diffusion seems to me not correct. Fick law of diffusion handles molecular diffusion with only the concentration gradient as driving force. In the case of methane in the North Sea I think that eddy covariance calculations would be more appropriate. The cited literature of Largier 2003, seems to be not appropriate as it is dealing with the distribution of particles and not dissolved molecules by advection!! and diffusion. There are some studies modelling the methane distribution in shallow sea, however they are using numeric modelling. Grunwald, M., Dellwig, O., Beck, M., Dippner, J. W., Freund, J. A., Kohlmeier, C., . . . Brumsack, H.-J. (2009). Methane in the southern North Sea: Sources, spatial distribution and budgets. Estuarine, Coastal and Shelf Science, 81(4), 445-456. Wahlström, I., & Meier, H. E. M. (2014). A model sensitivity study for the sea–air exchange of methane in the Laptev Sea, Arctic Ocean. Tellus B, 66, 24174.

Author reply 3: Fick's law is not only used for molecular diffusion, but also for eddy or turbulent diffusion in the ocean or large lakes. Wahlström, I., & Meier, H. E. M. (2014) used also turbulent diffusion and a reservoir to calculate the change of concentration over time and depth, similar to our approach; they wrote:

$$\frac{\partial \phi}{\partial t} + W\frac{\partial \phi}{\partial z} = \frac{\partial}{\partial z}\left(\Gamma_\phi \frac{\partial \phi}{\partial z}\right) + S_\phi \qquad (1)$$

where ϕ is the dependent variable (methane concentration in our case), t time, z vertical coordinate, W (m s^{-1}) vertical water velocity, Γ_ϕ (m^2 s^{-1}) the exchange coefficient (also named diffusion coefficient) and S_ϕ is the source and sink term for the dependent variable. The first term to the right represents turbulent diffusion and is according to Fick's first law. In this paper and also by Soetaert and Herman (A practical guide to ecological modelling, 2009, Springer) is stated that turbulent eddy diffusion applies not only for particles, but also for molecules.
The turbulent diffusion coefficient is much larger than the molecular diffusion coefficient (diffusion in water (molecular) ~ 10^{-11} – 10^{-7} m^2/s, vertical diffusion in the ocean, k*z* ~10^{-5}- 10^{-3} m^2/s, horizontal diffusion in the ocean, k*x* ~ 1 - 1000 m^2/s). Largier (2003) was cited as we used the horizontal diffusion coefficients reported in this paper. Largier wrote: "Further, a comparison of different studies indicates an exponential increase in *Ky* with distance from the shore (Fig. 3A): in wave-driven nearshore waters (*y*~0.1 km) values on the order of 1–10 m^2/s are found (Smith and Largier 1995), whereas values on the order of 100 m^2/s are found over the wind-driven shelf (*y*~10 km; Davis 1985, Largier et al. 1993), and much larger values on the order of 1000 m^2/s or greater are found in the offshore waters of the California Current (*y*~100–1000 km; Swenson and Niiler 1996).'
Eddy covariance is, as far as we know, applied in the atmosphere to calculate vertical turbulent fluxes within atmospheric boundary layers. This technique is also used to derive benthic oxygen fluxes

between seafloor and overlying water. Covariance means that two variables vary, wind and concentration vary in the atmosphere, oxygen concentrations and vertical velocity vary near the seafloor in the case of oxygen fluxes. Both variables are measured. What we have are vertical methane concentration profiles, but no measurements of vertical displacement. Therefore, we cannot use this technique. Furthermore, Sundermeyer and Price (1998) wrote: "Put another way, mixing processes are those which can (or must) be modeled by diffusion, i.e., molecular or very small-scale advective processes in which individual exchange events are not resolved, while stirring processes are resolved events, e.g., the streaking and folding of a tracer within a resolved eddy field."

Kinetics of MOx I cannot follow the conclusion that MOx rates are low. The MOs rates from this study lay well within the range of other marine areas, as the authors state. And even a comparison of the turnover times, which is independent from the M.conc. show that data from this study (100 d) are comparable with 80 - 1000 d from Gentz et al 2013, or 127 - 455 d from the Baltic Sea (Jacobs et al 2013). There are two ways of calculation k' the first order constant. It can be obtained via arithmetic, i.e. calculation the average or median of the single measurements or – as suggested by the authors – graphically i.e. the slope of the linear regression. However, if using the latter one has to prove / test the linearity of the relation and give as well the confidence interval of the regression line. But no matter how k' was calculated, it still will be only k' in the end.

Author reply 4: The reviewer is completely right that k' is still k' in the end, no matter how one derives the value. We could have also just taken the average of all k' and state the turnover time of 100 d to point out that the activity of methane oxidizing bacteria is low.
Our main point is that high MOx-rates do not indicate a highly active methane oxidizing bacterial community. If one has a k' of 0.01 d^{-1}, but different methane concentrations, e.g. 1000 nM and 1 nM, then one would get MOx-rates of 10 nM/d and 0.01 nM/d, respectively. The former suggests a fast consumption of methane whereas the latter a slow consumption, but actually the uptake rate of methane (or the activity of the bacteria) is the same.

The authors also use data from literature of the Km and a range of marine Mox rates to interpolate a Michaelis Menten kinetics. However, the methane concentrations of this study are in very low range and not even near the half-saturation concentration (1– 12M = 1.000 -12.000 nM). For a kinetic study to obtain vmax or Km much more data with a broader range of Mconc are needed. See also Lofton et al 2014. Thus I do not follow the interpolated values of vmax and Km given in the text and in figure 7.

Author reply 5: In Fig. 7A we show the range of kinetically possible uptake of methane by methanotrophs using Km and maximum MOx-rates published in literature. This figure shows that methane oxidation ranges from a rapidly increasing uptake of methane as soon as methane is available (MM1) to a slowly increasing methane-uptake although methane concentrations reach high values (MM2).
In Fig. 7B we fit a MM-curve to our data, that is, we adjusted K_m and v_{max} starting with the MM2-values until a good fit was reached. As noted in the response to the first reviewer having a related comment; the fitted curve has a R^2 of 0.81 in the range of our data. However, the reviewer is right that there is no proof beyond Km. Therefore, we rewrote the section and include a linear fit to the data assuming that we are in the linear range of the MM-kinetics based on the literature values (Page 15, Line 518). The linear fit gives a k' value of 0.01 d^{-1} with an R^2 of 0.82. However, this k' is based on 120 measurements instead of only replicates (n=2-3) or a time series (n=6-9). Therefore, k' is still k' but statistically more precise.

Please also note the supplement to this comment: http://www.biogeosciences-discuss.net/11/C8517/2015/bgd-11-C8517-2015-supplement.pdf

How much water was filtered? This would be important to know to explain the failure of the PCR to detect any methanotrophs.

Author reply 6: A similar comment was raised by reviewer 1. Here is our response: 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 samples was not as good as the quality of the summer samples, but from our experience with pelagic samples, the results are plausible, too.

I do not think that Ficks first law is valid here. It deals with molecular diffusion, assuming no turbulence or water movement. I think turbulent, eddy correlation would be more appropriate here. Furthermore, I do not see why this diffusion (if any) would only occur in two directions (west and east) but not north and south. So, the two dimensional assumption is difficult to support in a three dimensional water column.

Author: see reply 3 above

As I am not really convinced of the validity of the basic model (see above) I do not comment on the further application of this model.

Author: We described the validity of the equations in reply 3 above, which are the fundamentals of the model.

But in section 3.1 you state that there was no influence of the tides on the seepage intensity ????

Author reply 7: That is correct and the UWMS-data confirm this statement.

It would be nice to have a contour plot of the MOX-rates as well, for better comparison with the methane concentrations.

Author reply 8: Figure 8 shows the direct comparison of methane concentration and methane oxidation rates, thus, we think that a contour plot of MOx-rate data is not necessary. Moreover, we don't have a sufficient comprehensive data set of MOx-rates that would result in a reliable contour plot. Measurements were performed in five distinct water depth at each station and were partly excluded as some measurements were below the limit of detection.

But this is very strange if more methane is added to the sample, one would expect higher oxidation rate, but they seem to be lower than the 3H-rates??? I suggest omitting the 14C-data.

Author reply 9: We found similar results in Storfjorden, Svalbard (Mau et al., 2013). There, we suggested that the methane-oxidizing-community is not capable to consume more methane and, thus, cannot utilize the additional high methane load provided if using ^{14}C-CH_4 adding ~500 nM in contrast to adding about 2-3 nM in the case of ^{3}H-CH_4. In this paper, we further proposed that the use of ^{14}C-CH_4 as tracer allows to identify if methane oxidizing microorganisms in a water mass are capable to consume elevated methane concentrations or not.

This is an unexpected finding. Did the authors run positive controls to be sure that the PCR was working at all? How much water was filtered on the filter?

Author: see reply 6 above

But a lower seepage rate in winter would also lead to lower methane concentrations…

Author reply 10: Certainly, the seepage rate can be variable in time. However, the gas flare observations shown in the paper are observations of seepage in the winter that indicate an intense seepage although dissolved methane concentrations are low.

Figure 2: numbers for the clusters are much too small!, correct July 2013 (S12-20) and January 2014 (W2-12)

Author reply 11: We changed the number size and figure caption accordingly.

Figure 4: Are there 2 stations with 0 km? In the figure there are different sampling depth for the eastern and western transect. But three scales for 4 figures are too much. You could try with a log-scale or two scales with some data above or below the scale.

Author reply 12: Yes, there are two stations at 0 km as described in the method section. We corrected the different depth scales, but think that less scales for methane concentrations would decrease the information provided by the measurements.

Figure 5: It would be nice to show the MOx-rates in the same way as the Mconc in figure 3. Thus it would be more evident where the MOx-rates are elevated. As pointed out in the discussion, the

turnover time or k' are also important parameters, thus it would be nice to also depict them. The figure on the linearity of the incubation time is a basic assumption and could go to the supplementary info.

Author reply 13: Please refer to the reply 8 above for illustration of MOx-rates. We included a supplementary figure that shows the measured k' (Supplementary Material 6). We included the figure of the time series to show that only a small amount of the tracer was oxidized by microroganisms over four days suggesting a slow uptake of methane and confirming our hypothesis that microbial methane oxidation is a small sink of methane in the research area.

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

J. Kessler (Referee)
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The authors provide a straightforward and clean analysis of the methane dynamics at a relatively shallow seep site in the central North Sea. Methane concentration and oxidation rates are provided and used with some modeling investigations to constrain seasonal changes in mixing, oxidation, and air-sea flux. The manuscript finds that (1) summer stratification allows methane to accumulate in the deeper waters, (2) methane oxidation is a minimal sink of methane during all seasons, (3) there is likely an enhanced release to the atmosphere following the termination of summer stratification, and (4) dispersion largely causes methane concentrations to decrease.

My specific comments focus on two main issues: sampling/measurements and explanation of conclusions.

Sampling/Measurements: 1) I would have liked more explanation on the air-sea flux measurements. For example, was the concentration of methane measured in the atmosphere directly or are average atmospheric values used in your calculations? If you are collecting atmospheric samples for methane analysis, at what height about the sea surface are you collecting the samples? At what height above the sea surface did you measure wind speed and did you correct for a 10 m height?

Author: The atmospheric value was calculated using the Bunsen solubility and measured ocean temperature and salinities. Wind speed was measured 22 m above sea level onboard. We forgot to correct the value, but use dthe power law $V_h/V_{10}=(h/10)^{0.13}$ to do this. The corrected values are included in Fig. 8, but they do not alter our interpretation. Thanks for pointing out this issue (Page 9, Line 289-290).

Also, I'm slightly concerned about the possibility of under-sampling the surface waters for methane concentration. Is there a possibility that elevated dissolved concentrations of methane in the surface waters

were contained in fairly isolated plumes and thus you simply did not sample these regions? While using continuous, underway air-sea flux systems would have been nice (e.g. Du et al., Environmental Science & Technology, 2014) for a more comprehensive data set, a bit more discussion to justify that your discrete sampling was representative of this seep region would be helpful.

Author: Our intention was to compare the different fluxes. Although we have only a summer and winter snapshot picture based on a few discrete samples, still it is certain that e.g. wind speed is higher in winter and methane concentrations are lower in winter due to the enhanced mixing by wind. Therefore, the ratio of the flux estimates allow an evaluation of the importance of the different transport and loss processes of methane. A comprehensive data set such as the one you mentioned is needed to really quantify the sea-air flux and such an investigation would be necessary to be conducted at different wind speeds and covering a larger area. We included a more detailed discussion in a revised version (Page 17, Line 564-573).

2) The authors do not provide any data on current velocity. ADCP data would have been beneficial and would have helped to determine if turbulent mixing is significant. For example, on page 18023, line 7-8, the authors state that “The advective transport by ocean currents was not estimated as this process does not decrease the concentration of methane, but solely transports methane from the seep site in direction of the current flow.” That is only true if advective transport is uniform. Differences in current velocity and direction with depth would lead to turbulent mixing. These process may be contained in your vertical and horizontal eddy diffusion, but a bit more discussion on the magnitude and direction of the currents throughout the water column would have been beneficial.

Author: The second reviewer also suggested to present current measurements, although, as you pointed out, the turbulent mixing is included in the eddy diffusion. Unfortunately, we don’t have any ADCP-measurements, solely modelled data by the Bundesamt für Seeschifffahrt und Hydrographie (BSH) using wind and air temperature forecasts. However, we included a description and add a plot to the supplementary material showing the east-west and north-south velocities of the currents during the sampling campaigns (Page 10-11, Line 348-355). We also included an estimate of the advective transport (Page 8, Line 251-262, Page 16, Line 550-556).

Both of my comments on the “Sampling/Measurements” above are meant to provide clarity to the reader. I do not anticipate they will change the conclusions of this manuscript significantly.

Conclusions: 1) Perhaps the most impactful finding of this manuscript is that methane oxidation was not a large sink of methane. I am very curious to learn more about why this is, especially during summer stratification. Do the authors have access to nutrient or trace metal data to suggest why this may be? From a different perspective, what increase would the authors have expected in the average k’ value for methane oxidation during summer stratification? The lack of an increase in the k’ values for methane oxidation is a significant discovery but without any explanation as to why this is occurring makes the manuscript feel incomplete.

Author: Similar to the finding of Tavormina et al., 2013, who observed that there is not a strong correlation between methane concentrations and putative methanotrophs, we also find that there is no strong correlation between methane concentrations and activity of methane oxidizing microorganisms. We agree with these authors that either these putative methanotrophs are facultative methanotrophs, not necessarily dependent on methane, or that there is a limitation by e.g. trace elements, which are mainly limited in the upper ocean. We included such a statement for completion (Page 20, Line 693-697).

In conclusion, this is a strong manuscript that makes quality measurements of methane dynamics and does a meaningful interpretation of the data. I doubt that any my comments will lead to significant modifications to the conclusions in this manuscript. Nonetheless, they would provide a bit of clarity to the interested reader. I enjoyed reading this manuscript.

Seasonal methane accumulation and release from a gas emission site in the central North Sea

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Abstract

Hydroacoustic data document the occurrence of 5 flare clusters and several single flares from which bubbles rise through the entire water column from an active seep site at 40 m water depth in the central North Sea. We investigated the difference in dissolved methane distributions along a 6 km transect crossing this seep site during a period of seasonal summer stratification (July 2013) and a period of well mixed winter water column (January 2014). Dissolved methane accumulated below the seasonal thermocline in summer with a median concentration of 390 nM, whereas during winter, methane concentrations were much lower (median concentration of 22 nM) and punctually elevated due to bubble transport. High resolution methane analysis by an underwater mass-spectrometer confirmed our summer results and were used to document prevailing stratification over the tidal cycle. Although sufficient methane was available, microbial methane oxidation was limited during both seasons. Measured and averaged rate constants (*k'*) were on the order of 0.01 day^{-1}, equivalent to a turnover time of 100 days. Time series measurements indicated a microbial uptake of only 5-6% of the gas after 4 days, and no known methanotrophs and pmoA-genes were detected. Estimated methane fluxes indicate that horizontal transport rapidly disperses dissolved methane, vertical transport becomes dominant during phases of high wind speeds, and relative to these processes, microbial methane oxidation appears to be low. To bridge the discrete field data we developed a 1D seasonal model using available year-long records of wind speed, surface temperature and thermocline depth. The model simulations show a peak release of methane at the beginning of fall when the water column becomes mixed. Consistent with our field data, inclusion of microbial methane oxidation does not change the model results significantly, thus microbial oxidation appears

to be not sufficient to notably reduce methane during summer stratification before the peak release in fall.

1 Introduction

Methane is, after water vapor and CO_2, the most important greenhouse gas. Its concentration has increased by a factor of 2.5 since preindustrial times, from 722 ppb in 1750 to 1800 ppb in 2011 (IPCC, 2013). The total global emission was estimated to be ~550 Tg (methane) yr^{-1} with an anthropogenic contribution of 50 to 65%. Geological sources, which were not considered in IPCC reports previously, are suggested to account for up to 30% of total emissions and include anthropogenic emissions related to leaks in the fossil fuel industry as well as natural geological seeps both terrestrial and marine (IPCC, 2013). An improved emission estimate from marine seeps suggests that these sources contribute ~20 Tg methane yr^{-1}, i.e., 4% of the global emissions, to the atmospheric methane (Etiope et al., 2008).

In general, oceans have been found to be a minor source of methane to the atmosphere, accounting for 2-10% of the global emissions (Bange et al., 1994). A major fraction of the oceanic source (75%) is thought to originate from estuaries, shelf and coastal areas (Bange, 2006; Bange et al., 1994). For example, the European coastal areas were found to emit 0.46-1 Tg yr^{-1}, and thus contribute significantly to the overall global methane oceanic emissions (Bange, 2006). The author, however, points out that this estimate underestimates the coastal input, since fluxes from estuaries and shallow seeps are not adequately represented. Moreover, there is growing evidence that methane release from natural seepages and abandoned boreholes can significantly contribute to the global atmospheric methane emissions, especially from the North Sea (Judd et al., 1997; Rehder et al., 1998; Schroot et al., 2005).

It is important to consider shelf and coastal areas, as they are regions where most organic matter is deposited. Although continental margins account for only 10% of total ocean area and 20% of total ocean primary production (Killops and Killops, 1993), more than 90% of all organic carbon burial occurs in sediments depositing on deltas, continental shelves, and upper continental slopes (Berner, 1989). At these locations, which are also characterized by high sedimentation rates, organic carbon is rapidly buried beneath the sulfate reduction zone, and becomes available to methanogens (e.g. Cicerone and Oremland, 1988). Methane is also generated by thermal breakdown at high temperature and pressure. A significant fraction of the formed methane is oxidized in anaerobic and aerobic sediments (e.g. Boetius et al., 2000; King, 1992), the remaining methane may be transported into the overlying water either dissolved in upwardly advecting pore waters or in case of

oversaturation, in the form of gas bubbles. Because methane is undersaturated in seawater, rising methane bubbles partially dissolve during ascent through the water column (McGinnis et al., 2006), where the dissolved methane may be further consumed by microbial oxidation. Only if this methane survives transport to the mixed layer, can it be transferred to the atmosphere.

Using a bubble dissolution model in combination with acoustic observations of rising bubbles, McGinnis et al. (2006) showed that only bubbles emitted at shallow seeps (<100 m) may reach the atmosphere. Methane rich bubbles from deeper seeps fully dissolve in the ocean. Model simulations based on methane concentrations, oxidation rates, and current records of two plumes observed in the Santa Barbara Basin indicate that half of the dissolved methane reaches the atmosphere and the other half is microbially oxidized of the shallow plume whereas the deeper plume is mostly oxidized (Mau et al., 2012). Thus, depending on the emission depth, methane remains in the ocean and can be microbially oxidized.

Shallow seeps thus are likely more important contributors to atmospheric methane. However, even at shallow seeps, density stratification may limit the vertical transport. For example, at the 70 m deep Tommeliten area in the North Sea, less than ~4% of the gas initially released at the seafloor reaches the mixed layer during summer, because a seasonal thermocline constrains methane transport to the atmosphere (Schneider von Deimling et al., 2011). Summer stratification traps methane beneath the thermocline, some of which may be consumed by microbial oxidation, and some will be released in the fall during first storm events. In order to investigate the seasonal cycle of methane in the North Sea, we studied a shallow seep area both during summer (July 2013) when the water column was stratified, and in winter (January 2014) when the water column was well mixed.

1.1 Study Site

The study site is situated in an area of active gas venting above a shallow gas reservoir in the central North Sea. The gas vents are located in the Netherlands sector, license block B13 (Fig. 1). They occur at shallow water depth (< 45 m) in a flat region that lacks any morphological expression typical of seep structures (Schroot et al., 2005). These seeps are likely sourced from a biogenic methane reservoir ($\delta^{13}C$ values of -80‰ VPDB) of Pliocene to Pleistocene age, which lies 600-700 m below the seafloor. Patches of gas saturated sediments between the gas reservoir and the seafloor have been imaged in seismic data. These data plus observations of separate bubble streams in the water column and rapidly decreasing methane concentrations in cores with distance from the vent site led Schroot et al. (2005) to describe our study site as a leaking gas reservoir with laterally discontinuous seepage.

The seeps are located in the central North Sea, south of Dogger Bank, a sandbank with water depth 20 m shallower than in the surrounding sea. Water masses from the north (Atlantic Water) and south (Straits of Dover) meet (Kröncke and Knust, 1995) in this central area, where the general anticlockwise circulation along the coasts of the North Sea becomes weak and varied (Fig. 1, Howarth, 2001). The water above the sandbank is well-mixed throughout the year in contrast to the deeper waters surrounding the bank that become stratified during spring and summer. The front, where these waters encounter, bifurcates around the Dogger Bank and the location of the front is influenced by tidal current speed. Generally, tides have the strongest influence on currents in this region, followed by wind forcing (Howarth, 2001; Otto et al., 1990; Sündermann and Pohlmann, 2011).

Seasonal temperature stratification is a common feature in this and other shelf seas, and it separates high-light and low-nutrient surface water from low-light and high-nutrient bottom water. Even though in some shelf areas, the tidal energy is sufficient to overcome stratification, models by Pingree and Griffiths (1978) and Holt and Umlauf (2008) indicate that our study area is situated in a stratified region, east of the tidal front that surrounds the shallowest part of the Dogger bank. Thus, during spring and summer, the water column over the seeps investigated here, remains stratified over the course of a tidal cycle.

2 Methods

All data used in this study was collected during two cruises with *RV Heincke*. The first cruise (HE406) was conducted during summer 2013 (20.-24. July), the second cruise (HE413) during winter 2014 (13.-22. January).

2.1 EM710 flare imaging

During the winter cruise, we used a Kongsberg EM710 multibeam echosounder to map active gas emissions in the study area (Fig. 2). For the precise localization of individual flares, i.e., bubble streams in an echogram, the water column data were post-processed using the Fledermaus tools FMMidwater, DMagic, and the 3D Editor (© QPS). The origin of individual flares was identified as the point of highest amplitudes near the seafloor. The coordinates of these points were extracted using the FMGeopicker and subsequently plotted on top of the bathymetry using ArcGIS 10.2 (©ESRI).

For visualization of flare deflections and bubble rising heights, selected flares were extracted from the water column data as point data and edited using the 3DEditor of DMagic. The processed flares were plotted over the bathymetry data in a 3D-view.

2.2 Water column sampling

To identify the size and magnitude of the dissolved methane plume generated by the bubble discharge, seawater was sampled along a transect crossing the active gas emission sites (Fig. 2). The transect, which extends 3 km to the east and 3 km to the west from a bubbling location (cluster 1 in Fig. 2) was sampled twice, once in summer 2013 and once in winter 2014. In both cases, the eastern sector (5 stations) was sampled on one day (~3 h) and the western sector (5 stations) on another day (~3 h), so that the center stations was sampled twice.

Water samples were collected with a CTD/bottle rosette for methane concentration, methane oxidation rate, and molecular analyses. The rosette was equipped with twelve 5 L Niskin bottles, a Sea-Bird SBE 911 plus CTD, and an SBE 43 oxygen sensor for online monitoring of salinity, temperature, pressure, and dissolved oxygen. The data are archived in PANGAEA (doi:10.1594 / PANGAEA.824863 and doi:10.1594 / PANGAEA.832334). Twelve different water depths were sampled at each station for methane concentration analysis and 5 water depths for methane oxidation rates. Additional casts were conducted to sample sufficient water for molecular analyses.

2.2.1 Methane concentration

For methane concentration analysis, samples were collected in 60 ml crimp-top glass bottles. All sample bottles were flushed with 2 volumes of water and filled completely to eliminate bubbles. Bottles were immediately capped with butyl rubber stoppers and crimp sealed. After adding 0.2 ml of 10 M NaOH to stop any microbial activity, a 5 ml headspace of pure N_2 was introduced into each bottle as described in Valentine et al. (2001) and the samples were stored at 4 °C. One to two aliquots of the headspace were analyzed to determine methane concentrations using a gas-phase chromatograph equipped with a flame ionization detector. Analyses were performed both on board and post cruise. Replicate analyses of samples yielded a precision of ± 5%.

2.2.2 Methane oxidation rates

Methane oxidation (MOx) rates were determined from ex situ incubations of water samples in 100 ml serum vials. Sampling and incubations were performed as described in Mau et al. (2013). Briefly, duplicate samples were collected: the set of samples taken at all stations was treated with 50 µl of ^{3}H-labeled methane (160–210 kBq) in N_2, and a second sample set, which was collected at 5 stations in July 2013, was treated with 10 µl of ^{14}C-labeled methane (12–15 kBq). After shaking the bottles to equilibrate the tracer with the water, the samples collected in summer 2013 were incubated at 10 °C and those collected in winter 2014 at 9 °C. All samples were incubated in the dark for 24 h. After

incubation, the total activity (^{3}H-CH_4 + ^{3}H-H_2O) in 1 ml aliquots was measured by wet scintillation counting, and the activity of ^{3}H-H_2O was measured after sparging the sample for >30 min with N_2 to remove excess ^{3}H-CH_4. Incubations with ^{14}C-CH_4 were terminated by injecting 0.5 ml of 10 M NaOH. A 5 ml headspace was then added so that the remaining ^{14}C-CH_4 accumulated in the headspace, while produced ^{14}C-CO_2 and ^{14}C biomass was trapped in the aqueous NaOH solution. ^{14}C-CH_4 in the headspace was combusted to ^{14}C-CO_2, and ^{14}C-CO_3^{2-} was converted to ^{14}C-CO_2 through acidification with HCl. The produced ^{14}C-CO_2 was trapped in a solution of methoxyethanol and phenylethylamine, and the radioactivity was measured by wet scintillation counting.

MOx rates were calculated assuming first-order kinetics (Reeburgh et al., 1991; Valentine et al., 2001):

$$MOx = k'[CH_4] \quad (1)$$

where *k'* is the effective first-order rate constant calculated as the fraction of labeled methane oxidized per unit time, and [CH_4] is the in situ methane concentration. To verify first order kinetics we conducted time series incubations and measured the tracer consumption after 1, 2, 3, and 4 days.

In addition, control samples were frequently taken and poisoned immediately after the addition of the tracer. The mean ($\bar{x}$) and standard deviation (*s*) of all controls sampled during a cruise were calculated and the limit of detection (LOD) was set as:

$$LOD = \bar{x} + 3s \quad (2)$$

The *LOD* was 0.02 nM day^{-1} for the summer 2013 survey, 0.09 nM day^{-1} for the winter 2014 survey, and 0.0005 nM day^{-1} for the ^{14}C-methane survey in summer 2013.

The MOx values were also corrected for differences between in situ and incubation temperatures (Supplementary Material 1).

2.2.3 Analysis of bacterial communities

The composition of the bacterioplankton assemblages was examined using denaturing gradient gel electrophoresis (DGGE) based on the 16S rRNA gene as described in Mau et al. (2013). In short, immediately after sampling, 8 L of water were filtered and bacterial cells were concentrated on Nuclepore filters (0.2 µm pore size). The filters were stored on board at -20 °C and at -80 °C post

cruise. DNA was extracted by an UltraClean Soil DNA Kit (MoBio Laboratories, USA). 16S rRNA gene specific PCR was conducted using the forward primer GM5 plus GC-clamp and the reverse primer 907RM (Muyzer et al., 1993) under conditions described by Gerdes et al. (2005). The PCR products (ca. 500 bp) were analyzed by DGGE according to the protocol of Muyzer et al. (1993). Clearly visible bands of the DGGE gels were excised from the gel. The DNA was reamplified by PCR (Gerdes et al., 2005) and sequenced. The 16S rRNA gene sequences were taxonomically assigned by SILVA Online Aligner (Pruesse et al., 2012).

The presence of methane-oxidizing bacteria in the communities was checked by searching for genes encoding the particulate methane monooxygenase (*pmoA*), a key enzyme of methanotrophs (McDonald et al., 2008). The *pmoA*-gene-specific PCR reaction was conducted by using the primer set "pmoA" and amplification conditions described in McDonald and Murrell (1997).

2.3 Methane concentration analysis by underwater mass-spectrometry (UWMS)

In addition to the conventional methane analysis, in situ methane concentrations were detected and quantified with an UWMS (Inspectr200-200, Bell et al., 2007; Gentz et al., 2013; Schlüter and Gentz, 2008; Short et al., 2001; Wenner et al., 2004). The fast sampling frequency (≤ 2 s) of the UWMS allows mapping and quantification of methane in much higher resolution than the commonly used CTD/rosette-sampling technique. The instrument consists of a membrane inlet system (MIS), an Inficon (Bad Ragaz, Switzerland) Transpector CPM 200 quadruple mass spectrometer, a Varian (Palo Alto, USA) turbo pump, a roughing pump, a peristaltic pump (KC Denmark), an embedded PC, and a microcontroller. The UWMS was partly redesigned to include a cooling system (Ricor, K508), which lowers the detection limit for methane to 16 nM. The cooling system and the improvement of the detection limit are described in detail by Gentz and Schlüter (2012) and Schlüter and Gentz (2008). For reproducible gas permeation through the MIS, water is constantly heated to a steady temperature of 50°C and pumped at a flow rate of 3 ml min^{-1} along the membrane by an external peristaltic pump.

The UWMS was deployed above the central gas seeps (cluster 1, Fig. 2) on 21.07.2013 (16:31 – 22:32 UTC) at five different water depths: just above the seafloor, 35 m, 28 m, 25 m, and 10 m. When the system had reached the respective depth, the research vessel moved slowly along a rectangular transect (~125 m S-N, ~150 m E-W, Fig. 2) surrounding the flares of cluster 1 (4°5.44'N, 55°18.36'E) and towed the UWMS, which continuously measured the methane concentrations. Each of the depth transects took about an hour and recorded 400-800 methane concentration values.

2.4 Estimation of methane fluxes

Advection, horizontal and vertical turbulent diffusion, sea-air flux, and microbial oxidation were quantified for the upper (0-30 m) and lower water column (30-40 m) during summer stratification (July 2013) and for the entirely mixed water column (0-40 m) in winter (January 2014).

The advective flux (*ADV*) was calculated by multiplying methane concentration (*C*) and current velocity (*v*):

$$ADV = vC \quad (3)$$

Methane concentrations were averaged above and below the thermocline in the case of the summer results and throughout the water column in the case of the winter results. Current velocities refer to the resultant velocities calculated from the u and v component of the velocity vectors (Supplementary Material 2 and 3) and were averaged over the time period of sampling. The current data were provided by the *Bundesamt für Seeschifffahrt und Hydrographie* (BSH) (www.bsh.de/de/Meeresdaten/Vorhersagen/Vorhersagemodelle/index.jsp) and were modelled using wind and air temperature forecasts.

Turbulent horizontal and vertical diffusion (*Diff*) were calculated with Fick's first law of diffusion as described in Mau et al. (2012):

$$Diff = D\left(\frac{\partial C}{\partial x}\right) \quad (4)$$

where *D* is the horizontal or vertical diffusion coefficient in $m^2\ s^{-1}$. $\delta C/\delta x$ is the spatial concentration gradient in $nM\ m^{-1}$, estimated between the center and the outermost stations in the case of horizontal diffusion calculation, and the concentration gradient between the lower and upper water column in the case of vertical diffusion, calculated only for summer 2013.

D_h, the horizontal diffusion coefficient, can range between 0.1 and 1000 $m^2\ s^{-1}$ (Largier, 2003; Sundermeyer and Price, 1998) depending on the proximity to land. As the study area is located more than 230 km from shore, we used a D_h of 1000 $m^2\ s^{-1}$ for our calculations. The vertical turbulent diffusion coefficient (D_v) can vary between 10^{-3} and 10^{-6} $m^2\ s^{-1}$ depending on the energy in the water column (wind, tides, etc.) and stratification (Denman and Gargett, 1983; Wunsch and Ferrari, 2004). As a first approximation, we used 10^{-4} $m^2\ s^{-1}$, which is a common cited value across the thermocline. The vertical eddy diffusion was estimated for all vertical profiles (all 10 CTD-stations).

The sea-air flux was calculated as:

$$SAF = k_W(C_W - C_A) \quad (5)$$

where k_W is the gas transfer velocity in cm h^{-1}, C_W is the measured concentration of methane and C_A is the methane concentration in atmospheric equilibrium, both in nM. We calculated k_W, which depends on wind speed and the temperature-dependent Schmidt number of the gas, using parameterization developed by McGillis et al. (2001). Wind speed was recorded 22 m above sea level onboard and corrected to the standard height of 10 m. C_A was derived using the Bunsen solubilities given by Wiesenburg and Guinasso (1979) and measured ocean temperature and salinities. The sea air flux was calculated for surface water samples of all 10 stations sampled in summer 2013 and winter 2014.

The oxidative loss (OL) was calculated by depth integration of the MOx rates:

$$OL = \overline{x}_{MOx} z \quad (6)$$

where $\overline{x}_{MOx}$ is the averaged MOx rate in nM day^{-1} over the depth interval z in m. The depth interval is defined by the water stratification in the case of summer 2013 and covers the entire water depth in the case of winter 2014. Integration was done for all vertical profiles.

2.5 Seasonal model

A non-steady state, 1D-model was developed to investigate the temporal evolution of methane over a year. We considered an entirely mixed water column during the winter month, stratification development during spring that lasted until early fall when the entire water column becomes mixed again. Hence, we considered one water layer during fall-winter (0-40 m) and two layers (upper and lower water column) during spring and summer. The initial model configuration was defined by the dissolved methane concentration observed in January 2014 (17 nM, excluding punctual high concentrations due to bubbles) and the transport and loss quantities calculated for the mixed water column condition in this month. We set the methane flux from the seafloor to be equal to the *SAF* estimate of January 2014. In daily time steps, the *SAF* and the vertical eddy diffusion were calculated using Eq. 3 and 4 (above) and based on the amount of methane obtained in the previous time step. The parameters: mixed layer depth, wind speed, and surface water temperature were kept constant over a month, but then adjusted to the conditions of the following month. The mixed layer depth

was determined from archived CTD-profiles (Pangaea) collected in an area extending from 3-6°E and 54-56°N. Monthly mean wind speed was taken from the web-site: www.windfinder.com of the Ameland Oil Platform (mean of data from Aug. 2010-Mar. 2014) and Forties/North Sea (mean of data from Dec. 2012- Mar. 2014). Surface water temperatures were provided by the BSH.

3 Results

3.1 Seep locations

Echosounder data indicate bubble emission in the area of the sampled transect (Fig. 2). The center station was located at a known gas bubble emission site or flare cluster, where several bubble streams occur in close proximity to each other. We observed an additional four flare clusters near the western sector of the transect, which displayed a similar seepage intensity as at the central seep site. In contrast, no additional flares were found in the area of the eastern sector. Although echosounder data point to bubbles rising to, or close to, the sea surface, no bubbles were visually identified at the sea surface due to rough sea state in winter 2014, however, surfacing gas bubbles were visually documented when the sea was calm in summer 2013. Seepage intensity showed no obvious variation related to tidal cycles, i.e., pressure variations due to high or low tides, rather, seeps were found to be active during all survey crossings.

3.2 Oceanographic setting

In summer (July 2013) a seasonal thermocline separated surface (0-30 m) from bottom water (30-42 m; Fig. 3). The surface water consisted of a 10 m thick mixed layer below which the temperature decreased stepwise from 17.5 to 7°C in 30 m. Lower salinity was observed in 15 and 25 m depth, which departed from the general 34.55. The stepwise decrease in temperature and the salinity variations indicate the successive development of several pycnoclines driven by increasing sea surface temperatures and less wind activity in spring and summer. The oxygen concentrations increased from 220 µM at the surface to 240 µM at 30 m. In contrast to the surface water, the bottom water had a homogeneous temperature of 7°C, a salinity of 34.63 PSU and contained less oxygen (190 µM).

In winter (January 2014) the entire water column was mixed (Fig. 3). The water had a temperature of 7°C, a salinity of 34.85 PSU, a density of 27.3 kg m^{-3}, and oxygen concentrations of 280 µM.

Modelled current data indicate a dominant north-west transport with velocities ranging between 0.06 and 0.27 m s^{-1} (resultant velocity). In summer, the eastern part of the transect was sampled when currents were directed to the north-west with an average velocity of 0.24 m s^{-1} and the

western part was sampled when currents turned from north-west to south-west with an average velocity of 0.19 m s^{-1}. In winter, the eastern part of the transect was sampled when water moved north-east turning north-west with an average velocity of 0.22 m s^{-1} and the western part was sampled when water also turned from north-east to north-west, but with an average velocity of 0.1 m s^{-1}.

3.3 Methane concentrations

Consistent with the two layer structure observed by the temperature/salinity data, methane concentrations in summer 2013 also show a two layer distribution, with higher concentrations in the bottom water relative to the surface values (Fig. 4A). Methane concentrations in the surface water range from 3.9-517.8 nM with a median of 32.5 nM. Methane concentrations in the bottom water range between 39.7 and 1627.7 nM with a median of 390.6 nM. Highest concentrations in the surface water were found near the center station (170 nM), which decreased to the outermost stations (to the west to 96 nM and to the east to 13 nM). However, the decrease is not continuous due to the presence of bubble emission sites in the area. Similarly, in the bottom water the highest methane concentrations were found at the center station (600-700 nM) decreasing unevenly towards the outmost stations (200-300 nM). In both layers the methane concentrations exceed the background concentration of ~20 nM as measured at a reference station, which was located 32 km from the central station (Supplementary Material 4), and reported methane concentrations in Grundwald et al. (2009). Even this background value is already oversaturated with respect to the atmospheric equilibrium concentration of 2.3-2.9 nM (at the relevant T /S conditions, Wiesenburg and Guinasso, 1979).

In winter 2014, much lower methane concentrations were found (Fig. 4B). Highest values were observed near the center site with concentrations of up to 656.6 nM. But such high concentrations decreased rapidly horizontally (within 1 km) and were not encountered during repeated measurements at the same location. The median of all methane concentration measurements along the transect is 22.4 nM, which is only slightly above the regional background concentration. In general, methane concentrations indicate a patchy distribution as expected in an active seep area.

3.4 UWMS methane concentrations

During the cruise in summer 2013, the UWMS was deployed in the vicinity of gas flare cluster 1 (Fig. 2). Because the instrument was towed close to several bubble streams, the recorded methane concentrations range over three orders of magnitude, from 0 to 2127 nM in surface water (transects in 10 m, 25 m, 28 m) and from 259 to 2213 nM in the bottom water (transects in 30 m and 40 m) (Fig.

5). Nonetheless, the general pattern of lower methane concentrations in the surface and higher concentrations in the bottom water observed by conventional methods (see section 3.3) is also apparent in the UWMS-data. The median values of the records in 10 m, 25 m, and 28 m water depth range from 54 to 402 nM and in 30 m and 40 m depth, the medians range from 512 to 793 nM.

The UWMS measured the methane during ebbing tides, where water levels fell from 0.18 to -0.27 m, whereas CTD/rosette samples were collected during rising tides, when sea level height increased from -0.21 to 0.06 m and from 0.04 to 0.16 m (Supplementary Material 5). Again, the general pattern of lower concentrations in the surface and higher ones in the bottom water was apparent in all stations, even though methane data were obtained during different tidal phases.

3.5 Methane oxidation

Similar to the distribution of methane and co-located oceanographic data, MOx rates in summer 2013 show a two layer pattern whereas MOx measured in winter 2014 are uniform throughout the water column (Fig. 6 A, B). In summer, significantly less methane was oxidized in the surface water relative to the bottom water. In the surface waters MOx-rates ranged between 0.04 and 92.64 nM day^{-1} with a median of 0.10 nM day^{-1} and in the bottom water between 1.60 and 840.93 nM day^{-1} with a median of 3.99 nM day^{-1}. The total range of both layers (0.04- 840.93 nM day^{-1}) exceeds the range of MOx-rates observed during the winter survey (0.09-8.72 nM day^{-1}). The median of all MOx-rates measured in January 2014 was 0.24 nM day^{-1}.

Time series and ^{14}C-methane tracer incubations indicate a slow oxidation rate of methane over time. Although the methane concentrations greatly differ during both seasons, only 5-6 % of the ^{3}H-methane tracer was utilized during 4 day of incubation (Fig. 6D). In the ^{14}C-methane tracer experiments, a significantly higher concentration of methane is added to the sample relative to the ^{3}H-methane tracer additions (Mau et al., 2013). However, even the elevated methane additions did not lead to a higher methane utilization. The MOx-rates determined using ^{14}C-methane tracer additions range from 0.0009 to 0.04 nM day^{-1} with a median of 0.003 nM day^{-1} in the surface water (Fig. 6C). In the bottom water, the values range from 0.05 to 0.53 nM day^{-1}, with a median of 0.16 nM day^{-1}. Even though the ^{14}C-MOx-rates were lower than the ones obtained with the ^{3}H-methane tracer, in both cases the two layer structure was obvious for the summer 2013 situation.

3.6 Microbial communities

Molecular samples taken in summer 2013 show also a difference between surface and deep waters, whereas winter 2014 samples indicate a homogeneous distribution of microorganisms (Fig. 7, Tab.

1). In summer 2013, different DGGE banding patterns reveal the changes in microbial communities with depth. The surface water samples showed two strong bands (Fig. 7, bands 6, 7) that could be affiliated to the *Rhodobacteraceae* and two bands that could be assigned to the *Cyanobacteria / Synechococcus* clade (8, 9). The middle and bottom water samples were characterized by a strong chloroplast band (2), but also showed bands affiliated to the *Rhodobacteraceae* (5, 6). In the bottom water samples of the central station, we found an additional band, assigned to *Pseudoalteromonas* (10). The gel pattern of the winter samples showed no significant bands. The sequences of the faint bands excised were of low quality. Only two of the bands could be assigned to the *Rhodospirillaceae* (12, 13).

Neither the summer nor the winter bacterial communities exhibited known methanotrophic bacteria, even though the samples originate from an actively gas venting area. The absence of methanotrophic bacteria was further supported by the negative results of the pmoA-PCRs that targets a methanotroph molecular marker gene.

4 Discussion

4.1 Distribution of methane in summer and winter

Our highest dissolved methane concentrations measured in the bottom water reach magnitudes similar to those observed at other shallow seep sites (Tab. 2). Our highest value of 1627.7 nM is comparable to measurements near the Coal Oil Point seep field, Santa Barbara Basin, California (up to 1900 nM, Mau et al., 2012), and it is higher than methane concentrations reported for the Tommeliten, North Sea (268 nM, Schneider von Deimling et al., 2011), and offshore Svalbard, west of Prins Karls Forland (524 nM, Gentz et al., 2013).

Even though gas bubbling was observed at the sea surface in summer months, the dissolved methane at these and also at other vent sites is trapped beneath a thermocline or halocline, which hampers further ascent of the dissolved methane to the atmosphere. The studied seeps are located at a depth of 40 m and the dissolved methane plume was found beneath a seasonal thermocline. At the Tommeliten seep site, the methane plume was also observed beneath the seasonal thermocline (Schneider von Deimling et al., 2011) whereas the methane plume originating from the 245 m deep seeps offshore Prins Karls Forland was confined to water depths beneath a local halocline (Gentz et al., 2013). In the Baltic Sea, summer stratification also leads to accumulation of methane below the thermocline (Gülzow et al., 2013). At all these sites, an enhanced release of methane to the atmosphere is thought to occur upon erosion of stratification. In contrast, the dissolved methane plume originating from seeps situated between 5 and 70 m at the Coal Oil Point is dispersed above

the thermocline within the mixed layer (Mau et al., 2012), and as such is not controlled by seasonal stratification patterns.

Trapping and accumulation of dissolved methane beneath a thermocline is also well documented in lakes and freshwater reservoirs, where thermal stratification separates methane-poor, surface water from the methane-rich, but anoxic, bottom water in e.g. a shallow floodplain lake in south-eastern Australia (Ford et al., 2002), in a polyhumic lake in southern Finland (Kankaala et al., 2007), in the subtropical Lake Kinneret in Israel (Eckert and Conrad, 2007), and in eight freshwater reservoirs in India (Narvenkar et al., 2013). The accumulated methane is released when water starts mixing driven by enhanced wind forcing and lower temperatures.

Our results verify the assumption that in a seasonal stratified system, no methane accumulation occurs in winter, when the water column is well mixed as indicated by vertical profiles of temperature, salinity, and oxygen. During our winter field program, methane concentrations were found to deviate only due to bubble ascent and were otherwise low and constant throughout the water. The median winter concentration of 22 nM is similar to the background methane concentrations of 20 nM reported by Grunwald et al. (2009) for the German Bight, but the concentration is elevated relative to water originating from the Atlantic Ocean, which carries 2.5-3.5 nM of methane (Rehder et al., 1998) and to the methane background concentrations of <5 nM at Tommeliten (Niemann et al., 2005; Schneider von Deimling et al., 2011).

4.2 Low methane oxidation

Measured MOx-rates at our study site lie at the upper end of MOx-rates previously reported, which span over six orders of magnitude from 0.001-1000 nM day^{-1} (Tab. 2 and summarized in Fig. 1 in Mau et al., 2013). The rates measured in deep water samples during summer (median 3.9 nM day^{-1}, up to 840 nM day^{-1}) equal those observed in the Gulf of Mexico after the Deepwater Horizon event (median 10 nM d^{-1}, up to 820 nM day^{-1}) (Valentine et al., 2010). Even winter time rates are high in comparison to rates measured in the Eel River Basin, an area of hydrate dissociation (Valentine et al., 2001) and match rates of the Coal Oil Point seep field in the Santa Barbara Basin (Mau et al., 2012; Pack et al., 2011).

However, we note that in spite of the reported high MOx values, detailed analysis of the data reveals an overall low activity of methane oxidizing microorganisms. This apparent contradiction arises from the fact that the MOx-rate of a given sample is traditionally calculated by multiplying methane concentration with the fraction of the tracer converted per unit time, i.e., *k'* - the first order rate

491 constant. At a given *k'* value changes in methane concentrations yield MOx-rates that are low or high
492 depending on whether methane concentrations are low or high. Thus high MOx-rates might just
493 reflect high methane concentrations, and not necessarily a rapid turnover rate. The constant *k'*
494 provides an indication of the relative activity in a water sample (Koschel, 1980), but it cannot be
495 viewed independently from methane concentration, as *k'* is derived from tracer conversion in a
496 sample with ambient methane concentration.
497
498 Alternatively, the MOx-rate can be plotted against methane concentration, following the approach
499 used by Michaelis Menten (MM) kinetics to describe the rate of a first order enzymatic reaction that
500 depends on one substrate, by relating the reaction rate (*V*) to the substrate concentration (*S*) (Fig. 8).
501 The model takes the form of the equation:
502
503 $$V = v_{max} \frac{S}{(K_m + S)} \quad (7)$$
504
505 where v_{max} is the maximum uptake rate and K_m is the concentration at which the reaction rate is half
506 of v_{max}. As illustrated in Fig. 8, the enzymatic uptake can be very rapid as soon as methane is available
507 and levels off when enzyme saturation is reached (low K_m and high v_{max}, MM-kinetics 1). However, in
508 some systems the uptake can be very slow, and enzyme saturation is reached at very high methane
509 concentrations (high K_m and low v_{max}, MM-kinetics 2). K_m values of cultured and uncultured soil
510 methane oxidizing bacteria range between 0.8-12 µM (Baani and Liesack, 2008; Bender and Conrad,
511 1993). For v_{max}, we used MOx-rate maxima reported for oceanic environments, which range between
512 100-1000 nM day^{-1} (Mau et al., 2013). Using these wide data ranges, we depict the predictive
513 behavior using both end-member for MM kinetics. Apart from 7 data points, which were collected in
514 the bottom water close to flare cluster 1 (stations S12 and S13, Fig. 2), all other data points are close
515 to a curve that follows MM-kinetics 2, with high K_m value and low v_{max}, hence pointing to a generally
516 slow uptake and oxidation of methane.
517
518 As all methane concentrations of our data are below K_m, we can derive an overall *k'* value from the
519 slope of the linear regression (Fig. 8), which for our case is 0.01 day^{-1}. As expected this value matches
520 the majority of the measured *k'* values (median of summer data: 0.02 day^{-1}, median of winter data:
521 0.01 day^{-1}, Supplementary Material 6) as well as the value *k'* derived from the time series incubations
522 (0.01 day^{-1}, n=4). We pose that, rather than using MOx-rates, or *k'* values from individual samples, a
523 fit to the entire data set provides an effective way to generate an overall parameter *k'*, which best
524 reflects the ecosystem microbial activity. The inverse of the modelled *k'* gives a turnover time of 100
525 days suggesting a rather low activity of methane-oxidizing bacteria in both summer and winter.

The low activity of methane oxidizing microorganisms is further supported by time series experiments, ^{14}C-methane spike experiments, and molecular analysis of filtered matter from seawater. Time series incubations show a slow uptake of methane over time, solely 5-6% of the added ^{3}H-methane-tracer was converted after 4 days. Even when we spiked the sample with elevated ^{14}C-methane concentrations of 400-500 nM, there was no additional substrate utilized after incubation for one day, indicating that methane oxidizing microorganisms cannot rapidly consume the additional methane. Consistently, DGGE and *pmoA* analysis did not reveal the presence of any known methanotrophic bacteria or *pmoA*-genes. Either methanotrophs were only present in low numbers and/or poorly matched to the used PCR primers and, thus, were not detected (Hansman, 2008). Other observations in shallow marine waters (< 200 m) in the Pacific, Atlantic, and the Gulf of Mexico show also that canonical methanotrophs were not detectable, but revealed novel sequences closely related to those coding for methane monooxygenase (Elsaied et al., 2004; Tavormina et al., 2008; Tavormina et al., 2013; Valentine, 2011; Wasmund et al., 2009), an enzymatic hallmark of aerobic methanotrophs.

4.3 Transport is faster than methane oxidation

Although a part of the methane flux to the atmosphere is supported by direct bubble-transport, a component that is being constrained by video observations and gas bubble samples (T. Gentz, personal communication, 2014), here, we focus on the fate of the dissolved methane fraction. When methane enters the water column, it is transported by ocean currents and spreads by horizontal and vertical eddy diffusion. Dissolved methane can then support methane oxidizing microorganisms and if water with methane concentrations higher than saturation reach the mixed layer, methane will be transferred into the atmosphere. In order to evaluate the relative importance of these transport and loss processes, we estimated the advective transport, the horizontal and vertical eddy diffusion, sea-air flux, and integrated the MOx-rates over the water depth (see methods). All fluxes were estimated in units of nmol m^{-2} s^{-1}. As shown in Fig. 9, we estimated summer fluxes for the bottom (30-43m) and surface waters (0-30 m), using data collected in July 2013, and winter fluxes for the entire unstratified water column (0-42m) using data from January 2014. The results show that advective transport and the horizontal eddy diffusion are the dominant processes rapidly transporting and diluting the emitted methane. The loss processes, i.e., sea air flux and microbial oxidation, are more than 4-orders of magnitude lower than these horizontally directed processes. Our flux estimates revealed that in summer more methane is transported via vertical diffusion into the surface water than is oxidized in the bottom water. In the surface water, 50% is oxidized and the other 50% is transferred into the atmosphere. In winter, the sea air flux removes more methane from the water

column due to increased wind speed. Overall the flux estimates indicate that diffusion (dilution of the methane rich water with background ocean water) outcompetes microbial methane oxidation.

All of these flux estimates are snapshots based on a few discrete samples and may vary by up to one order of magnitude. The estimates were determined as described by Mau et al. (2012), which includes a detailed discussion of the uncertainties associated with the calculations. Briefly, the uncertainty originates from the precision of the different measurements, assumed diffusion coefficients, and the parameterization of the gas transfer velocity. The uncertainty does not include any possible variations during the 3 h of sampling. However, as the overall environmental setting will remain constant, e.g. with the establishment of a seasonal thermocline and higher wind speed during winter, the trend indicated by the flux comparison persists despite the uncertainties. Advection and horizontal diffusion of methane will remain consistently higher than vertical diffusion and methane oxidation.

Our flux estimates suggest that microbial oxidation is of minor importance in the central North Sea. Particularly during periods of high wind speed (fall and winter), more methane reaches the atmosphere than is oxidized in the water. In summer when lower wind speeds prevail, methane oxidation is similar in magnitude to the gas transfer to the atmosphere. Our findings are similar to those reported by Scranton and McShane (1991), who conclude that methane oxidation constitutes a relatively small sink for methane in the Southern Bight of the North Sea (0.00023-0.3 nM day^{-1}), relative to methane losses to the atmosphere (0.00026-7.5 nM day^{-1}), which are highest during periods of high wind speed. The data are consistent with estimates for the shallow Coal Oil Point methane plume in the Santa Barbara Basin (Mau et al., 2012). There, 0.05 mol day^{-1} are oxidized in the surface water and 0.03 mol day^{-1} are transferred to the atmosphere.

4.4 Modeled methane accumulation and flux to the atmosphere over a year

To extend our inferences based on 2 field programs to seasonal changes over an entire year, we developed a 1D model using wind speed, sea surface temperature, and the depth of the mixed layer defined by the depth of the thermocline. CTD data of the surveyed region were used to specify the monthly development of the mixed layer depth, which develops in May and deepens until the entire water column becomes mixed in September (Fig. 10D). The model focused on the sinks of dissolved methane: sea air flux and microbial methane oxidation.

Three simulation were run. The first simulation included solely the vertical transport (sea air flux and vertical eddy diffusion during stratification), the second tested the uncertainty of the first simulation

due to the most unspecified parameter, *Dv*, and the third simulation included the microbial methane oxidation (Fig. 10A-C).

Model results of the first simulation, which do not include methane oxidation, illustrate the seasonal changes in methane concentrations. With decreasing wind speed in spring, methane concentration slowly rise in the water column; at the onset of stratification, most of the dissolved methane accumulates in the bottom water, leaving the surface water as the only source of methane to the atmosphere and, thus, reducing the methane concentrations in the surface water. As the concentration gradient between bottom and surface increases, more methane is transferred to the surface water by vertical eddy diffusion; that in combination with lower wind speeds in the summer cause methane concentrations to increase in the surface water. In late summer, beginning of fall, the mixed layer depth deepens due to increased wind forcing. Surface and bottom waters become mixed, which leads to a peak in methane concentration in the entire water column. This is transferred to the atmosphere by sea air exchange. Due to prevailing high wind speeds in fall and winter, methane concentrations rapidly decrease to a background concentration level of 20 nM.

The first simulation is greatly dependent on the vertical diffusion coefficient *Dv*. This parameter could be one order higher (10^{-3} m^2 s^{-1}) due to shallow water depth or one order lower (10^{-5} m^2 s^{-1}) due to low wind speed especially in summer (Denman and Gargett, 1983; Wunsch and Ferrari, 2004). For example, during the first sampling period in July 2013 a wind speed of 2-5 m s^{-1} was recorded whereas the average value used for the month July in the model was 7 m s^{-1}. Therefore, we tested the uncertainty of the model that results from the variability in *Dv* in the second simulation. The results of these simulations show that the modelled trend would be exaggerated if transport is less (*Dv* = 10^{-5} m^2 s^{-1}). That is, e.g. a larger methane peak is predicted at beginning of fall, which would be smoothed if we were to use a higher *Dv* (10^{-3} m^2 s^{-1}). The best fit to the data is achieved using a *Dv* of 10^{-4} m^2 s^{-1}, which yields a methane concentration of 39 nM in the surface water for the month July, similar to the median of the measurements, 33 nM. A methane concentration of 260 nM is predicted for the bottom water, which is equivalent in magnitude to the median of our measurements, 390 nM.

In order to further evaluate this critical parameter, we estimated *Dv* according to the equation by Osborn (1980):

$$Dv = Kz = \Gamma \frac{\epsilon}{N^2} \quad (8)$$

where Γ is the efficiency of mixing and assumed to be a constant of 0.2. We used published dissipation rates of turbulent kinetic energy (ϵ) in stratified shallow shelf seas (Palmer et al., 2008; Thorpe et al., 2008) and calculated the buoyancy frequency (N) from the available CTD-profiles. The results indicate that *Dv* is in the order of 10^{-4} to 10^{-6} m^2 s^{-1} during stratification. However, this rough approximation neglects hourly changes that can be already of one order of magnitude. For example, Palmer et al. (2008) observed and calculated *Kz* to range between 10^{-4} and 10^{-5} m^2 s^{-1} over a tidal cycle.

After obtaining an appropriate *Dv* (10^{-4} m^2 s^{-1}), which fits best to our methane concentration data, we included methane oxidation in the third model simulation. For this, we subtracted the averaged measured MOx rates from the surface and bottom water reservoirs. MOx was included in the surface water all year round, but in the bottom layer (i.e., in case of stratification) MOx was considered only when sufficient methane has accumulated, that is, for the month May we assume a negligible MOx in the bottom water. The model results do not show any significant difference by comparison to the first simulation (which included only transport) except for the month of June, when the model predicts significant methane consumption by MOx in the bottom water. Due to this decrease of methane concentration in the bottom water, the concentration difference between the surface and bottom water is not as large anymore and less methane is transferred by vertical eddy diffusion into the surface water. The simulation drives the concentration gradient to equilibrium, until the water column becomes fully mixed. We note that the model predicts a similar quantity of methane released to the atmosphere at the beginning of fall whether or not methane oxidation is included in the simulation.

In summary, if *Dv* is below 10^{-4} m^2 s^{-1} over the entire stratification period, then a peak release of methane occurs at the beginning of fall when the water column becomes mixed. Microbial methane oxidation appears insufficient to significantly reduce methane before the gas is transferred into the atmosphere.

The model does not take into account any temporal changes of methane emission. Furthermore, a sensitivity analysis of the model shows that especially wind speed affects model results. 10% higher or lower wind speed over the entire year would in- or decrease the annual flux by 28% or 23%, respectively. Sea surface temperature is less influencing the model result with 1.5% change if the temperature is 10% higher or lower throughout the year.

5 Conclusions

1. Observations at a shallow gas seep site in the central North Sea document methane accumulation below the thermocline during summer stratification, but no methane accumulation in the winter. Similar summer time results are presented by Schneider von Deimling (2011) for the Tommeliten area in the northern North Sea.
2. Our seasonal model bridges our summer and winter field studies and predicts an enhanced sea-air flux at the end of the stratification period. Such an elevated sea-air methane transfer was measured in the Baltic Sea when wind forcing increased after the summer month, breaking down the stratification (Gülzow et al., 2013). The seasonality in fluxes highlights the importance of understanding the effect of seasonal changes on estimates based on short field programs.
3. We show that MOx rates alone cannot be used to characterize the ecosystem microbial activity, as these values are scaled to the methane concentration. We instead propose the use of an average *k'* value of all the data, as an indicator of microbial activity. Such derivation generates a more realistic parameter than values based solely on replicate samples and is similar to values obtained by work-intensive time series incubations.
4. The idea that trapping of methane in the bottom water makes it more available to microbial oxidation could not be verified. Even though the residence time of central North Sea water is about 1.5-2 years (Prandle, 1984; Ursin and Andersen, 1978) and thermal stratification prevails for 4 months and could provide sufficient time to establish a methanotrophic community, we were not able to identify these organisms in the water column. Doubling times of planktonic marine methanotrophs are not known to the authors, as few if any such methanotrophs are currently available in culture. However, if we assume a doubling time of ~10 h as known from cultured methanotrophs (Baani and Liesack, 2008; Khadem et al., 2010) or a doubling time of 3.5 days as was estimated after the Deep Water Horizon incident in the Gulf of Mexico by Kessler et al. (2011), a methanotrophic community could potentially develop in the central North Sea. Even if the doubling time of methanotrophs in the field was even longer as nutrients and substrates can be limiting, still the residence time of the water would permit growth. Possible limitations of essential trace elements or that the methane oxidizing microorganisms are facultative methanotrophs (Tavormina et al., 2013), i.e., not necessarily depending on methane, might explain, why stratification over a summer season of 4 months does not enhance methanotrophy sufficiently to significantly hamper methane release to the atmosphere upon water column mixing.

Author contribution

S. M. designed study, measured methane concentrations and methane oxidation rates, calculated the fluxes, developed the model and carried out model simulations, wrote the manuscript

T.G., R. M., and M.S. deployed the UWMS and post-processed the data

J.-H. K., M. R., H. S., and P. W. collected and post-processed hydroacoustic data

M. T. interpreted methane oxidation rate data, edited manuscript

E. H. implemented and interpreted molecular analyses

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Tables

Tab. 1 Classification of partial 16S rRNA gene sequences (Fig. 7) to bacterial taxa performed with the Silva classifier (Pruesse et al., 2012). The confidence value (0–1) for assignment at the level of class and genus is given in parentheses.

No.	Class	Family
1	Alphaproteobacteria (0.4)	*SAR11 clade* (0.2)
2	Cyanobacteria (1)	*Chloroplast* (1)
3	Alphaproteobacteria (1)	*Rhodobacteraceae* (1)
4	Bacteroidetes incertae sedis (0.43)	*Marinifilum* (0.4)
5	Alphaproteobacteria (1)	*Rhodobacteraceae* (1)
6	Alphaproteobacteria (1)	*Rhodobacteraceae* (1)
7	Alphaproteobacteria (1)	*Rhodobacteraceae* (1)
8	Cyanobacteria (1)	*Synechococcus* (1)
9	Cyanobacteria (1)	*Synechococcus* (1)
10	Gammaproteobacteria (1)	*Pseudoalteromonadaceae* (1)
11	Proteobacteria (0.36)	
12	Alphaproteobacteria (1)	*Rhodospirillaceae* (0.8)
13	Alphaproteobacteria (0.91)	*Rhodospirillaceae* (0.7)

925 Tab. 2 Comparison of highest methane concentrations, methane oxidation rates, and sea-air fluxes from different locations

Location	Methane concentration	MOx-rate	SAF	Reference
	up to nM	*nM day^{-1}*	*nmol m^{-2} s^{-1}*	
Seep sites				
central North Sea	1628	0.04-840	0.02-8.3	this study
Coal Oil Point, Santa Barbara Basin	1900	0.02-30	1.8	Mau et al., 2012; Pack et al., 2011
Tommeliten, North Sea	268		10.8*	Schneider von Deimling et al., 2011
west of Prins Karls Forland, Svalbard	524	up to 0.8		Gentz et al., 2013
Eel River Basin	300	0.002-0.8		Valentine et al., 2001
Deepwater Horizon event				
Gulf of Mexico	180000	up to 820		Valentine et al., 2010
Gulf of Mexico	1000000	up to 5900		Crespo-Medina et al., 2014
Overall areas				
Baltic Sea	38		0.008-0.2	Gülzow et al., 2013
Southern Bight of the North Sea	372	0.0002-0.3	0.07-7	Scranton and McShane (1991)
general European shelf estimate	21		0.11-0.24	Bange, 2006
Lakes				
floodplain lake in south-eastern Australia	50000		8.3-2700	Ford et al., 2002
polyhumic lake in southern Finland	150000	30-14400	0.5-695	Kankaala et al., 2007
the subtropical Lake Kinneret in Israel	450000			Eckert and Conrad, 2007
freshwater reservoirs in India	156000			Narvenkar et al., 2013

*direct transport via bubbles

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927

928 Figures

929 Fig. 1: Location of the study area in the central North Sea. The main currents are shown following
930 Howarth (2001). The map was drawn using GeoMapApp with 40 m contours.

931

932 Fig. 2: Overview of gas flares mapped in January 2014 and CTD stations sampled in July 2013 (S12-
933 S21) and January 2014 (W2-W12). Flares cluster in 5 distinct areas (cluster 1-5) and reach to 6 m
934 from the sea surface (e.g. cluster 2 in upper right insert), which corresponds to the echosounder's
935 transducer depth. Hence, most likely the gas transport extends to the sea surface. Cluster 1
936 corresponds to the gas seep area investigated by Gentz (2013) (lower right insert).

937

938 Fig. 3: Depth profiles of potential temperature, salinity, density (sigma theta), and oxygen for all
939 stations in both summer and winter field programs.

940

941 Fig. 4: A-B Contour plots of the dissolved methane concentrations measured in the water column in
942 July 2013 and January 2014. The 6 km transect was divided into an eastern (positive numbers) and
943 western part (negative numbers) starting from the center station at 0 km. Note the different
944 methane concentration scales, which are necessary to properly display the different concentration
945 ranges. The black dots indicate the sampled water depths.

946

947 Fig. 5: Box plot of methane concentrations recorded by UWMS on 21.07.2013. The times on the right
948 side refer to the start and end times of the rectangular transects the UWMS was towed along in the
949 vicinity of flare cluster 1 (Fig. 2) at each water depth. Profiles obtained with UWMS are consistent
950 with discrete water sampling data.

951

952 Fig. 6: A-C Methane oxidation rates versus water depth measured with ^{3}H-methane in July 2013 (A),
953 with ^{3}H-methane in January 2014 (B), and using ^{14}C-methane as tracer in July 2013 (C). D Time series
954 of water samples collected during both field programs and incubated with ^{3}H-methane.

955

956 Fig. 7: DGGE profile of 16S rRNA gene fragments of samples from different depth and stations in the
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961 (MM-kinetics) using the parameters v_{max}=1000 nM day^{-1} and K_m=800 nM for curve MM-kinetics 1 and
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963 range of the enzyme kinetics available. B Close up of the data for MOx-rates < 20 nM day^{-1} and MM-
964 kinetics 2 in that range and the linear regression of all data (R^2=0.82).
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966 Fig. 9: Sketch of transport and loss terms estimated for the study area in nmol m^{-2} s^{-1}.
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968 Fig. 10: Model results over the course of a year. The mixed layer depth (D) shows the time period of
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971 surface (B) and bottom water (C) whereas during the rest of the year the entire water column is well
972 mixed with methane concentrations shown in B. A displays the sea-air flux based on monthly mean
973 wind speed derived from the stations shown in Fig. 1. Model simulations including solely vertical
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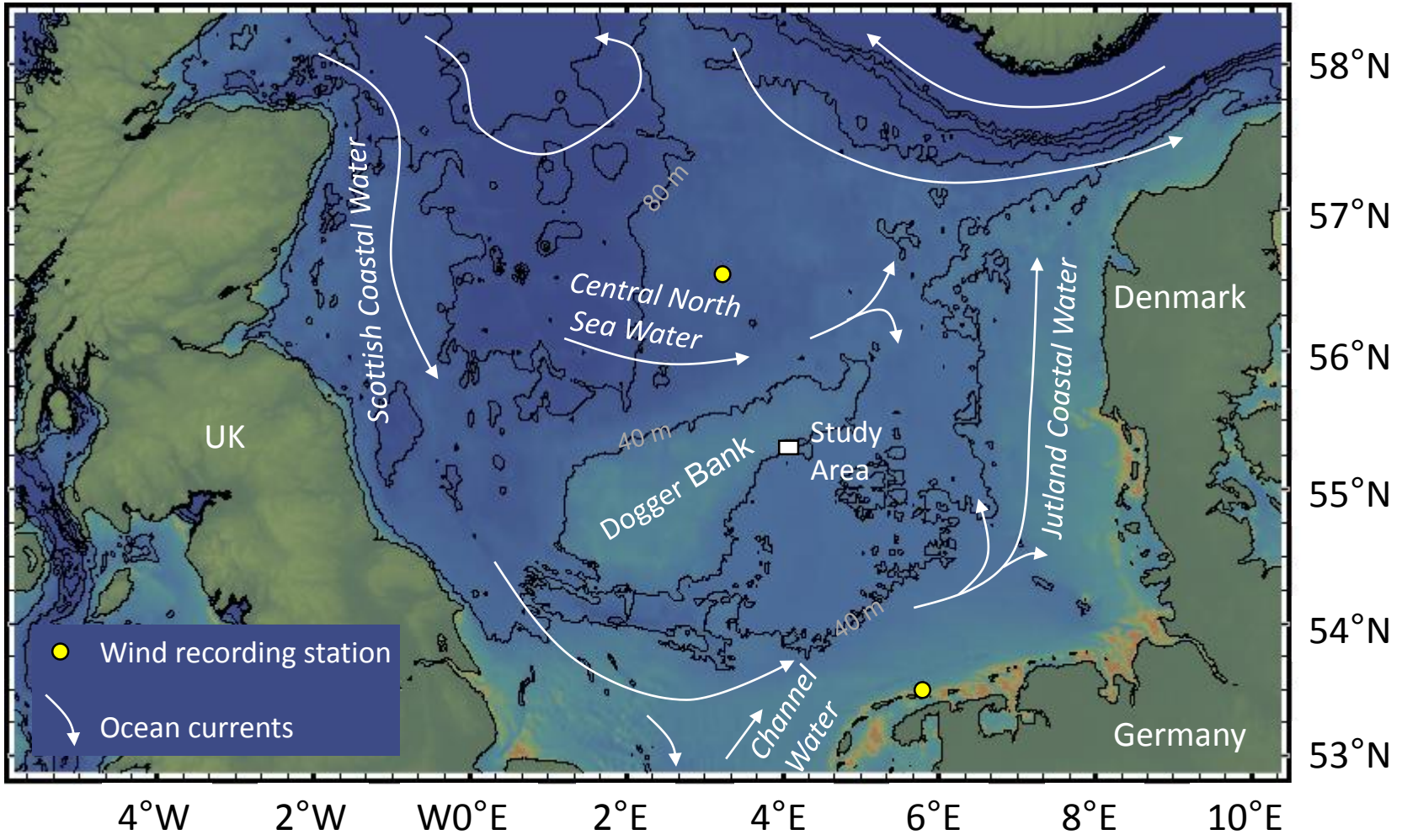
976 is shown as a red line.



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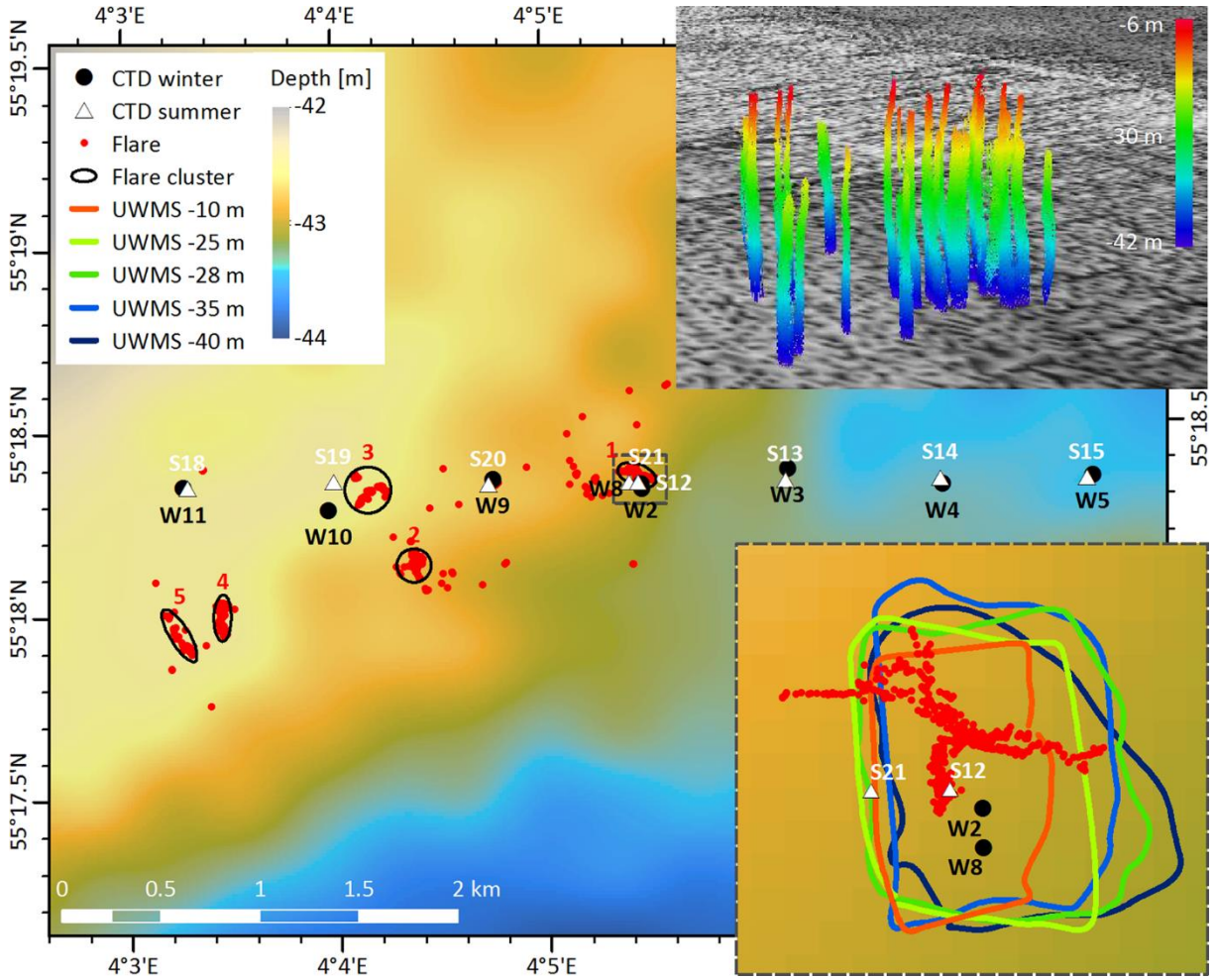

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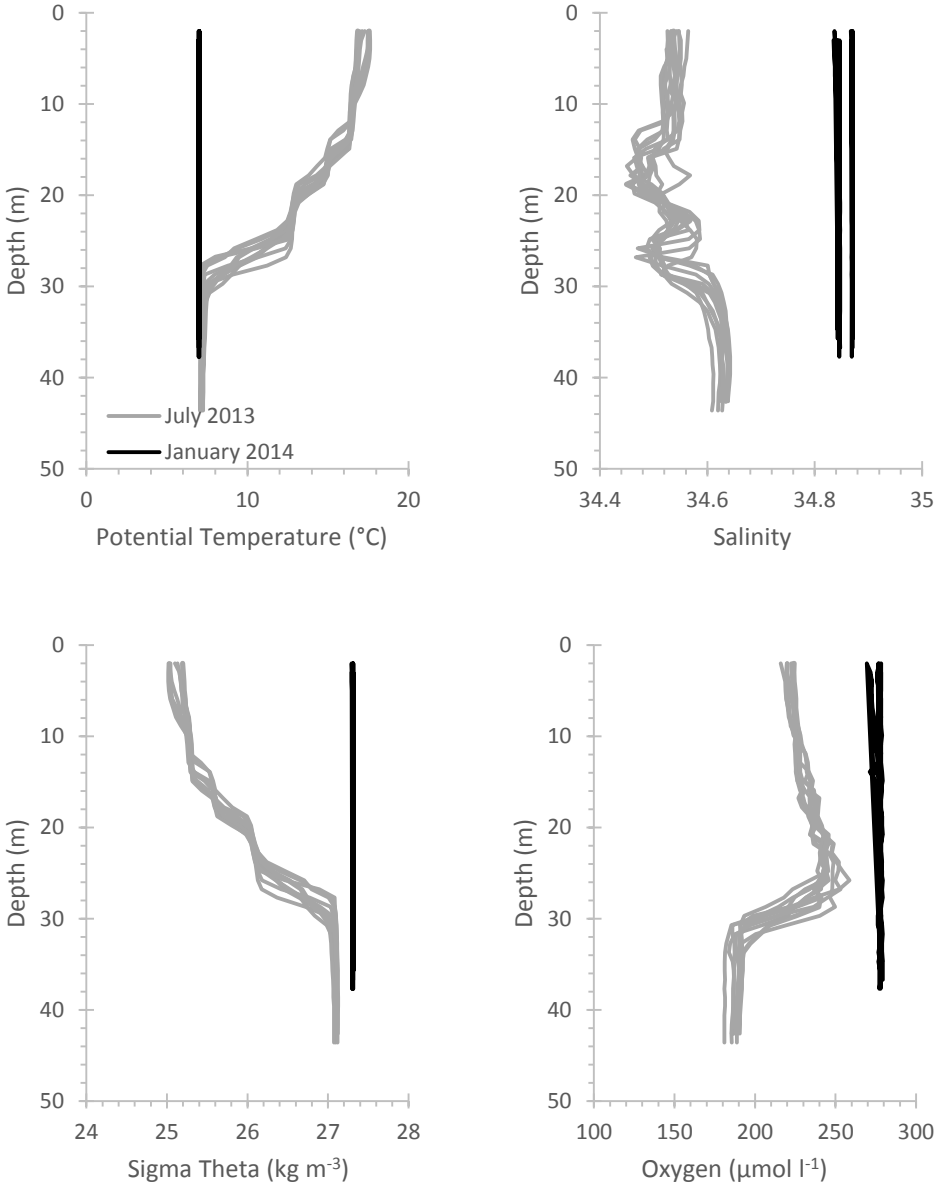

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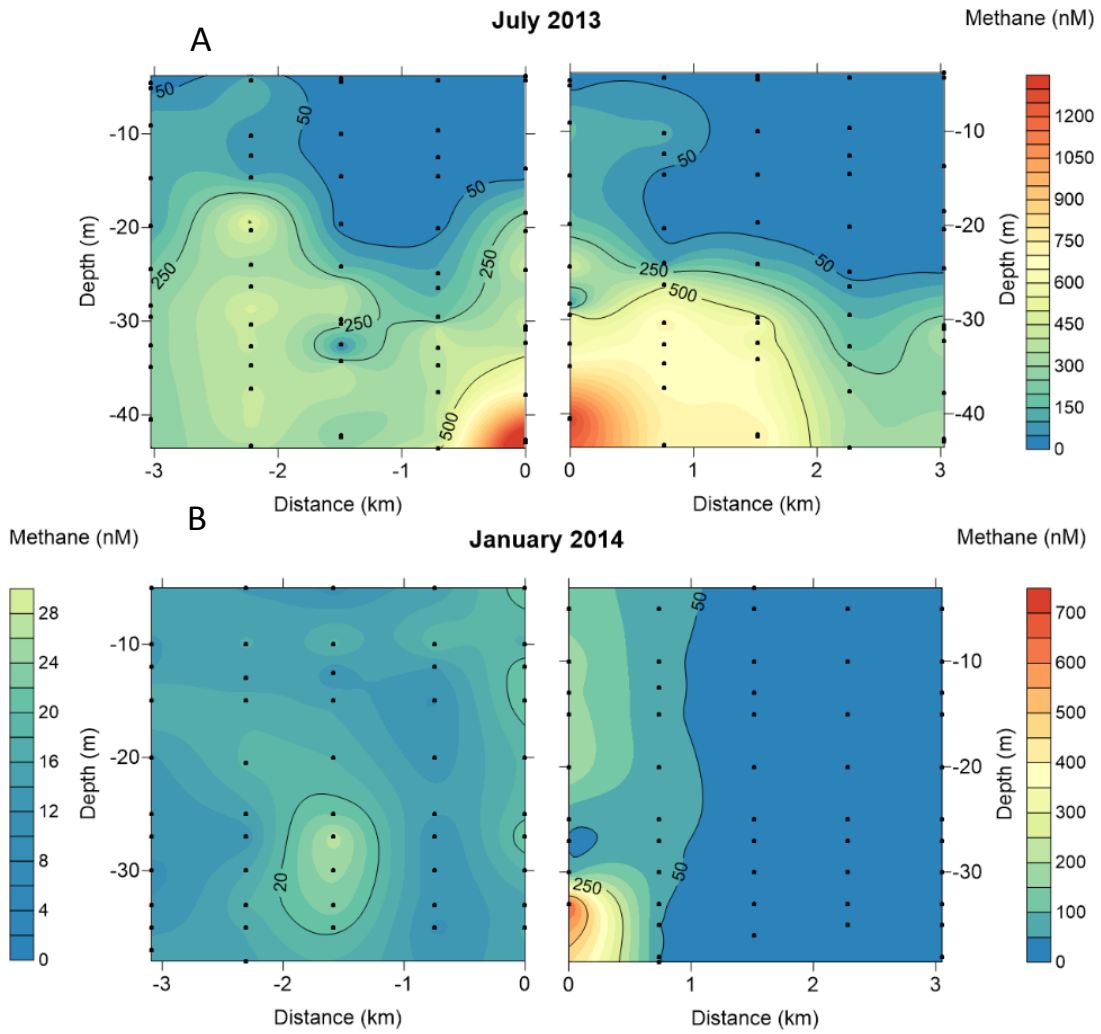

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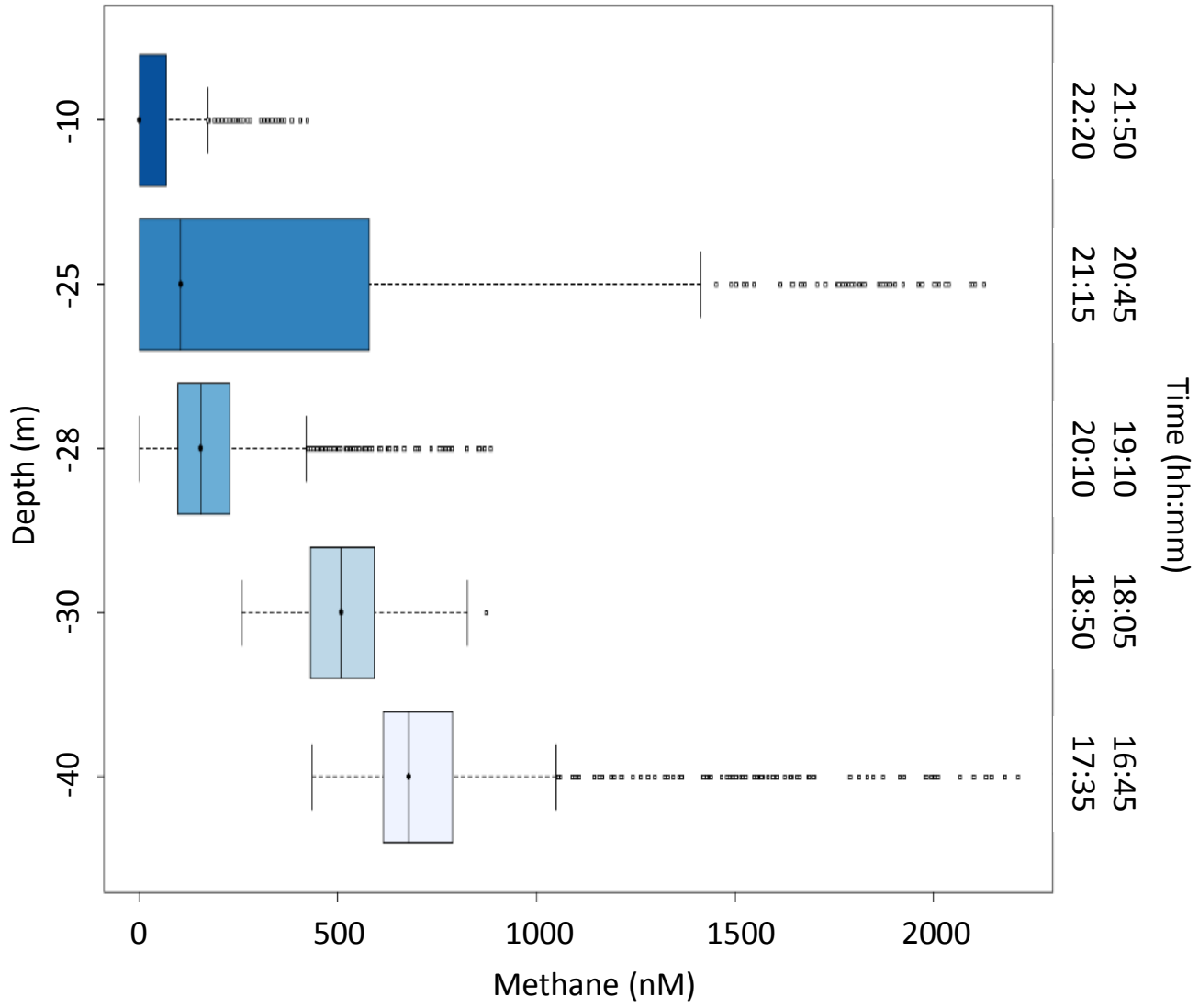

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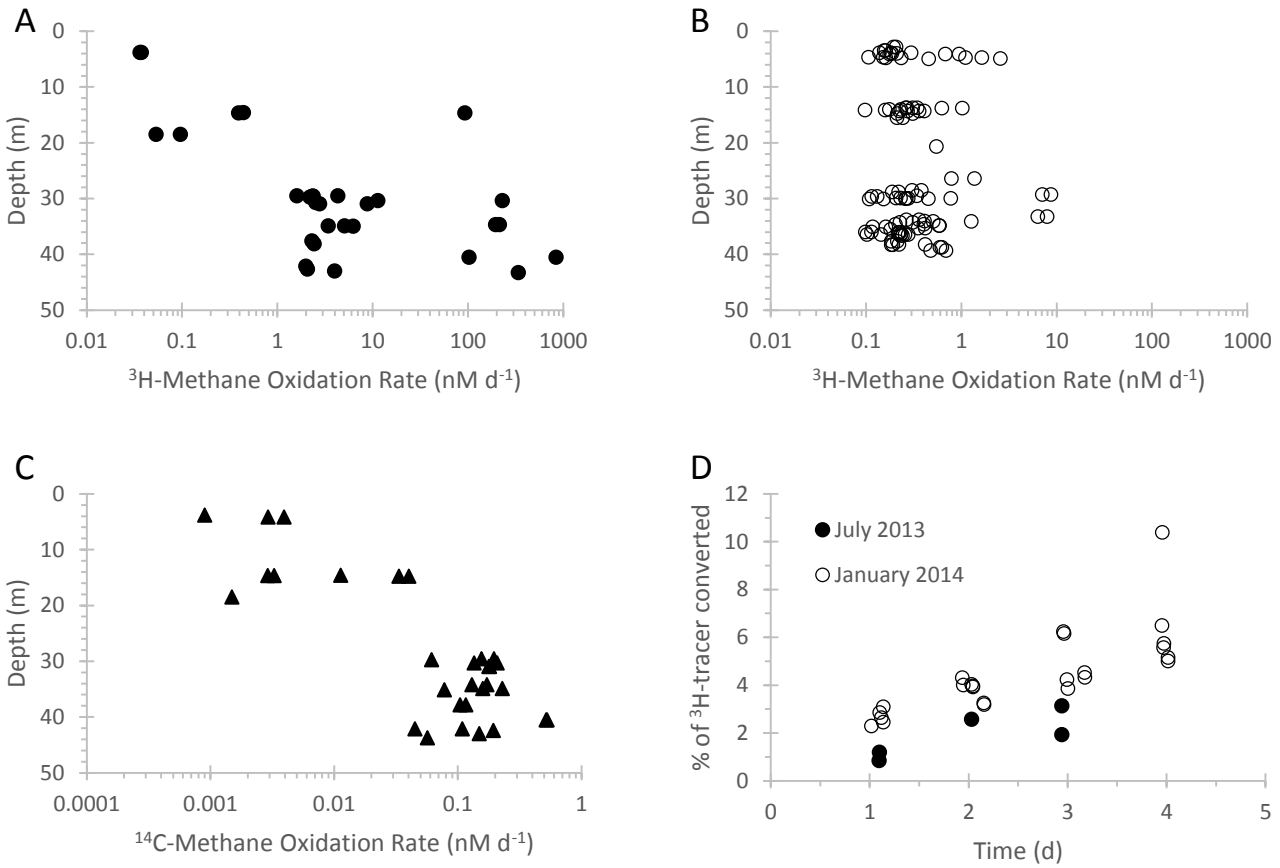

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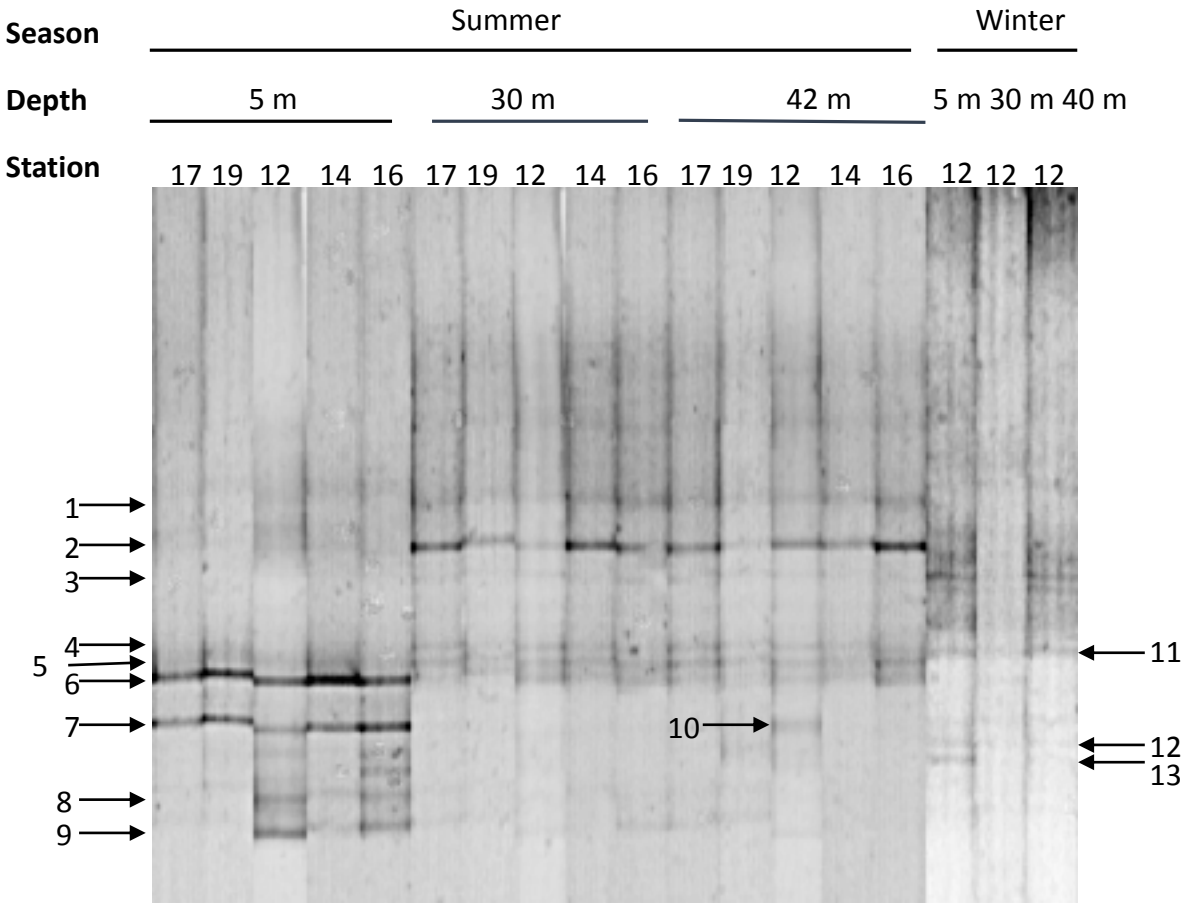

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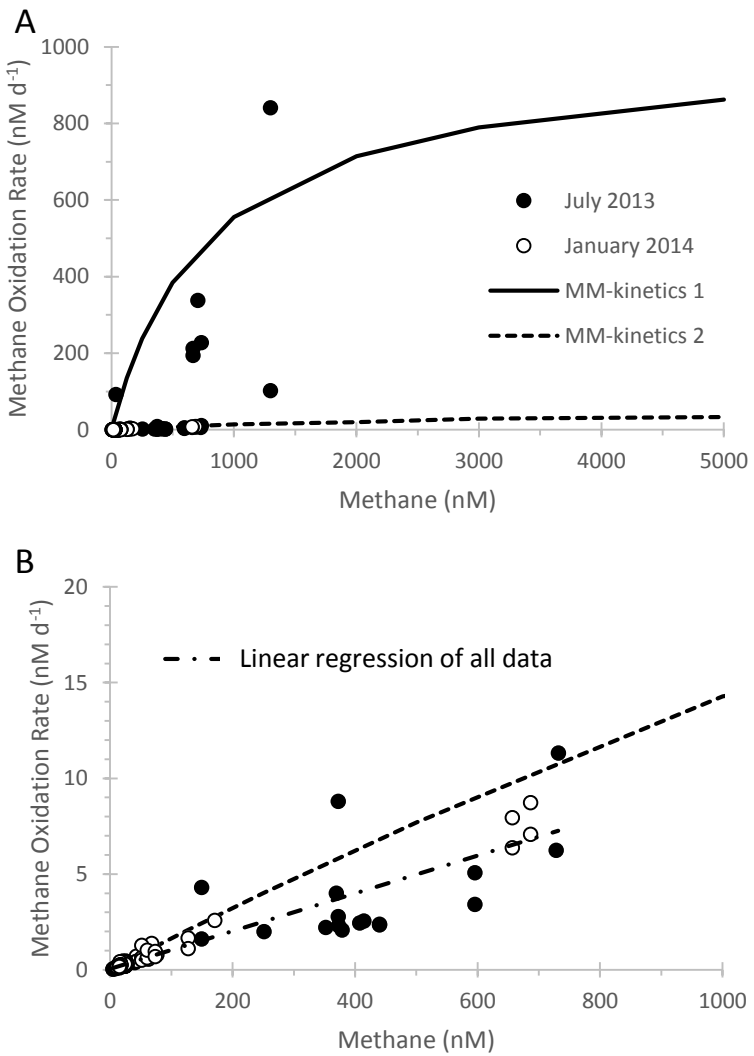

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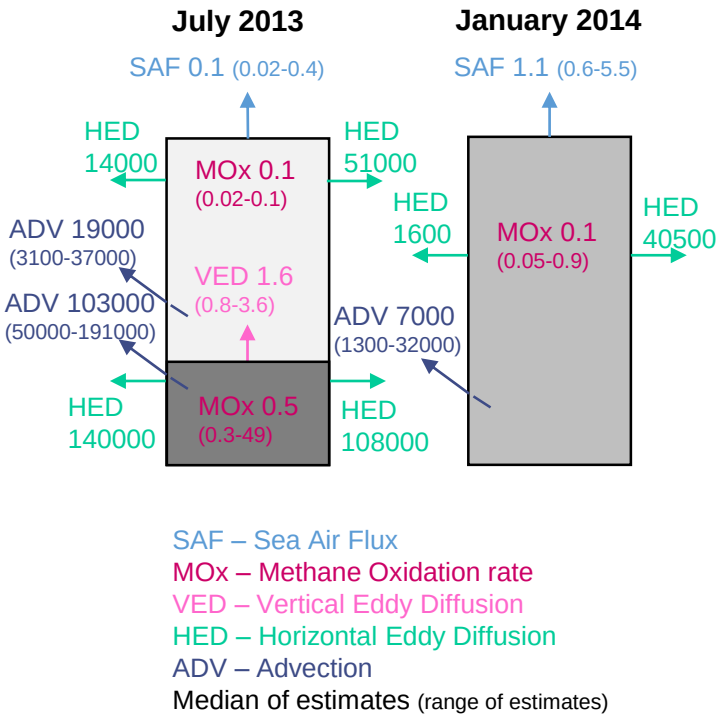

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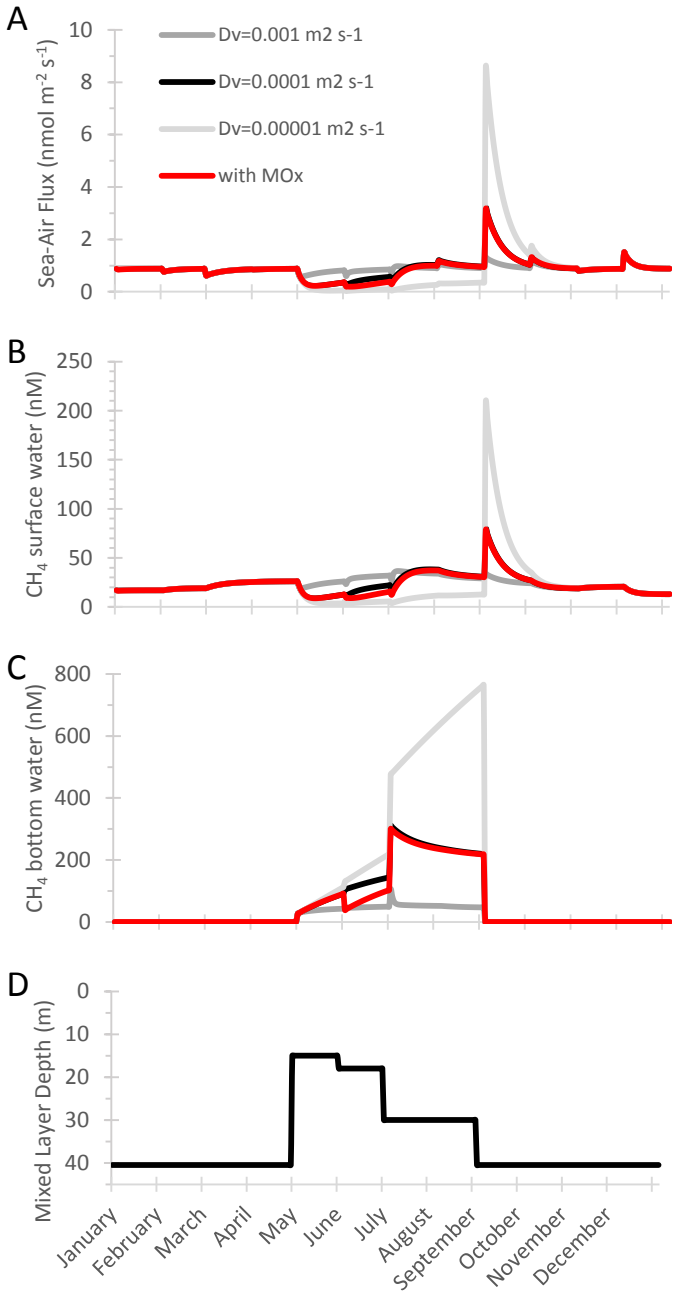

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