Author’s response

“Annual variability and regulation of methane and sulfate fluxes in Baltic Sea estuarine sediments” by Joanna E. Sawicka and Volker Brüchert

Anonymous Referee #1

1.) General Comments

The paper investigates the effects of changes in temperature, benthic oxygen concentration and eutrophication on the sediment concentrations and fluxes of methane (and sulfate) in two sites in the Baltic Sea, an open-water coastal site and a eutrophic estuarine site over 4 time points (spring, summer, fall and winter) within a 12-month season. In order to address this, the authors measured methane and sulfate concentrations, oxygen uptake and sulfate reduction rates and calculated sulfate and methane fluxes in the sediment in the upper approx. 45 cm of the sediments. The main influence on methane emission from the sediment was found to be by bottom water oxygen enhancing aerobic carbon mineralization and oxidative recycling of sulfate. The authors state that the seasonal changes in sediment methane concentrations are too large to be only the result of changes in methane generation and oxidation. Thus, they suggest advective recharge of methane from deeper, gas-rich sediment layers as possible influencing factor. The methane concentration below the sediment surface is lowered by AOM below the saturation concentration and thus bubble emission does not play a role at the investigated sites. The study presents a well-designed experimental set-up and the experiments are performed thoroughly.

However, the authors fail to formulate a clear scientific objective to conduct this research. It should be clear from the abstract and from the introduction why this study was conducted and what the expected merit would be. The abstract describes the findings and ends with the conclusion but it does not clearly mention the scientific questions addressed. At the beginning, the importance of this study should be made clear to attract the reader attention and interest, e.g. by naming the research question behind. Such questions can then be answered by the findings.

The introduction is well written, describes the state of the art and highlights some gaps in knowledge to justify the study. It also briefly summarizes the methods applied in the study. However, a concise statement what the presented study will contribute would make the paper sound much stronger. As mentioned above, it would be good if the authors state what problem they exactly address and how they do it – in other words, what exactly do they want to find out by the applied methods.

Answer: We reformulated our objectives and scientific aims and emphasized the scope of the study somewhat clearer.

The presentation of the results is confusing. There is major work needed to check the consistency of the figures and the text (see specific comments below). This makes it hard to follow the argumentation as one cannot relate the described results to the profiles. When presenting the results, I would suggest sticking to the same order of the stations throughout the entire manuscript. For example, always describe station B1 first and then station H6 and have the same order also in the tables and figures (i.e., B1 on top and H6 below). The whole section should be rewritten with a focus to guide the reader clearly through the graphs. More attention should be paid to the general consistency in the style of units. For example: mM vs. mmol/L vs. mmol L-1 (or e.g. nmol cc-3 d-1 and mmol L-1 OR nmol/cm3/d and mmol/L).

Answer: We have followed these suggestions to improve the clarity. See editions in Results section.

I suggest combining Figure 2 and 3 by plotting CH_4 and sulfate concentration in the same plot with linear concentration scale also for methane. The logarithmic scale for methane makes it hard to follow the changes and it is easily to compare with the sulfate profile if both are on the same scale and together. I also suggest showing all data of the triplicates for the sulfate reduction rates in Figure 4 and making the fit - not only from the medians (see details below).

Answer: We followed the recommendation and combined Figures 2 and 3..

The interpretation and argumentation as well as the conclusions seem reasonable and are well written. The conclusion contains many good arguments and statements of which I think it would be good to mention these in the abstract to raise the interest of the reader.

I suggest publication of this interesting study in Biogeosciences, however, I indicated major revision because the results presentation needs some careful rewriting with better guidance for the reader as well as careful cross-checking of text and figure/table content.

I think it is worth to add than 3 key words, to help finding the paper.
Keywords: Methane cycling, coastal and estuarine sediment, seasonality

2.) Specific Comments

Lines 30-32: rephrase the sentence, and maybe split. At the moment it says that "The effects of temperature [: : :] where **(A: where ?)** investigated [: : :] for open-water coastal and [: : :] sediment." That is probably not what the authors wanted to say.
Answer: We simplified this sentence, but we are not exactly sure what the reviewer meant by this comment.

Line 68: I would delete "summer"
Done

Figure 1: is of rather bad quality (at least in the document I could print out). It is impossible to read the names of rivers, cities or islands. Maybe the colors could also have more contrast to make the whole picture look sharper. A color code/legend could be helpful to understand the different blue tones (is this water depth?). If this differentiation is not important, a single color for water would be better.
Thanks for the suggestion. We have produced an alternative map in Ocean Data View, however, without the rastered high-resolution bathymetry. We would have liked to retain the bathymetric information, because it is important to understand the sediment deposition pattern, but cannot provide it at an overall satisfying resolution, because the bathymetric data are rastered for a large coastal area and cannot be presented as high-resolution cutouts for Himmerfjärden.

Lines 127, 129, 133, 148/149 : name equipment manufacture here
Okay. Done
Lines 180 – 185: total reduced inorganic sulfur should be abbreviated with TRIS, at least it should be consistent with the formula.
Okay. Done

Line 189: I think it is better not to use the median here. Out of three measurements (triplicates) the median will always be the measurement in the middle. This means your plot

and the input for the mathematical fit only relays on the one measured result (although there is the information behind that there is one higher and one lower measurement). You might talk about mean values in the text but in general I think you should present all individual measurements and also plot all data in the plots in Figure 5. And then you can calculate a fitted curve and also include this in the plot to visualize trends. It might be that the individual measurements show outliers and individual replicates differ. However, this is not uncommon for rate measurements and the best strategy is to simply show all data. Otherwise it could make the impression that something was tried to hide behind the median.
A: The format was chosen to present the trends in the best possible way. We have revamped the figures and now show all data points.

Line 245/256: be consistent in the order of described results (e.g. B1 as first and H6 as second) within the text for all parameters and also with table 1.
We carefully checked and revised the text to be consistent in the sequence the stations are listed.

Line 262ff: Please indicate the individual figure numbers after each station and result, e.g., “August... at station H6 (Fig. 2f) and : : :at station B1 (Fig. 2b) and so on for all mentioned data, this helps to identify the results in the figures. Please also make the order consistent over the entire manuscript. Moreover, here are some inconsistencies between text and figure that could be easily sorted out by referencing to the respective profile. For example, in the text is says highest CH_4 concentrations in August (H6: 5.7 mM, B1 1.9 mM). While for Station H6 (Fig. 2f) this might be true, for station B1 (Fig 2b) the figure I cannot see the 1.9 mM, in fact August 2012 has the lowest methane concentrations. Also in February, only at H6 (Fig, 2h) the CH_4 concentration is lowest, but not at B1 (Fig. 2d). However, the number mentioned in the Text for B1 Feb 2103 (0.1 mM max.) matches the highest values in the B1 Feb 2013 figure (Fig. 2d) but it is not the lowest CH_4 concentration in B1, this is in August 2012 (Fig. 2b).
Thanks for this suggestion. We adapted the changes to the text accordingly.

I am wondering about the use of a logarithmic scale for the methane concentrations, this is unusual for the presentation of sediment methane concentrations. The mentioned linear increase in sediments at H6 is not visible due to the logarithmic concentration scale and also not the described “concave upwards trend” for B1. Also the mentioned differences in maximum concentrations are not visible due to that scale. Here, a linear concentration scale would be better to visualize the concentration changes. It would furthermore allow for a better judgement of the data quality and the efficiency of the sampling protocol (in terms of potential methane loss). A linear scale would also be helpful to compare the data with the sulfate data and the maximum sulfate penetration depth indicated by the green line. When using the linear scale it could be a good idea to combine Methane and Sulfate Profiles (Figures 2 and 3) in one plot for each sampling point.

A: We have reconverted the figures back to a linear scale. We were well aware of the potential criticism the use of a logarithmic scale can draw. However, logarithmic scales have been used frequently, e.g., in the IODP literature. Here the main purpose was to convey the scale of the concentration change and to demonstrate that the sampling technique allows us to capture concentrations above the concentration at atmospheric pressure with consistency, but that the measured concentrations were consistently far below the saturation concentration at in situ pressure. The linear scale does not allow us to do this. However, we welcome the honest opinion of the reviewer, and therefore reverted to the conventional linear scale.

Line 269: “concave upwards trend” what is meant by this? This is very unusual for a profile description. Do you mean increase followed by decrease? Here also a linear concentration scale would help to understand.

A: Scale was changed back to linear to avoid more confusion.

Line 272ff: I do not see that the sulfate concentration gradient at station H6 in October 2012 (Fig. 3g) . For me it seems that the steepest increase is in August (Fig.3f) (>6 mM over < 10 cm depth)

A: The steepest gradient occurs between 6 and 10 cm depth in October whereas the surface gradient is less steep. This is likely due to the increased O_2 concentrations and the colder temperature in the fall leading to a downward propagation of a less steep sulfate gradient, however, not yet at a depth below 6 cm.

Line 275: better : “At station H6, sulfate was always fully depleted within the cored sediment interval, : : :”
A: okay, changed

Line 276: “Depletion already occurred at 5 cm depth in April and October and at 9 cm in August: : :” Depletion occurs all the way down from the surface sediment to the lowest concentration in the profile. Do you mean complete depletion (or depletion until a low constant level)? This is at approx. 9 cm depth at H6 in August 2012 (Fig. 3f) but I cannot see the 5 cm in April (Fig. 3e) and October (Fig. 3g), or do you refer here to Station B1 (Fig. 3a-d)?
A: The reviewer is correct. We changed this.

Figures: 2-3 It would be helpful to quickly identify the profiles mentioned in the text, if station number are indicated, e.g., for each row. The letters (a), (b), etc., should be larger in order to better overview the figure and relate it to the text while reading. The style of the units should be consistent with the format used in the text (mmol/L vs. mM). As mentioned in the comments above, I suggest combining figures 2 and 3 and presenting the methane concentration with a linear scale.
A: We have increased the numbers and assign the station names to each row. We also plot sulfate and methane together in one plot, Figure 2

Line 287 ff: also here, please indicate the related profile in the figure 4 always directly when mentioned in the text, to help the reader understanding the text quickly.
A: Done

Line 288/289: I don’t see an SRR increase to 63 nmol cm-3 d-1 in any of the profile of B1. The maximum SRR I see is in Fig. 4a at approx. 35 nmol cm-3 d-1. Also for station H6, I do not find a maximum of 411 nmol cm-3 d-1 in the figure. The maximum measured is around 350 nmol cm-3 d-1 in Fig. 4e. Are these the individual measurements (i.e. from one of the triplicates?) As mentioned above, I suggest showing the data of all triplicate samples. If you refer to theoretical values at the very surface calculated from the regression line, please indicate so.

A: We now show all replicates and show the regression lines based on the power law model. The author is correct in that assuming that the reference in the text was made for the individual measurements and not the averages.

Line 305: What is the “peak between 6 and 9 cm depth? Isn’t that a second peak? Sulfate is already mostly depleted at 10 cm and CH4 seems to be at maximum concentration below 10 cm. Could this increase SRR her not indicate AOM? Again, an overlay of sulfate and methane concentrations profile with linear concentration scale (combined Figs 2 and 3) would help to judge this better.

A: We had of course also debated the second peak. It is a consistent feature, since it is also nicely visible in the 3 replicate SRR. However, there is no change in the sulfate gradient or the methane gradient in this depth intervals that would be expected if the measured rates would be largely attributable to AOM. Therefore, we consider it less likely that the SRR at this depth indicate largely AOM and interpret this peak as due to organiclastic SR.

Figure 4: please indicate what H6 and B1, in the caption and best also in the Figure itself (e.g. for each line of plots). As mentioned earlier, I would like to see all individual data here instead of the median.
A: Done.

Figure 5: A separation line between the four sampling times would be helpful for a better readability.! Maybe also indicate them with Letters and reference to the plots in the text when described. Add the error bars if the errors are mentioned in the text. The figure says February 2013 but the Table January 2013 Line 314/315 and Fig 5: In the fig 5, highest TOU at H6 is in April (_33 mmol m2 d-1) and at B1 in August _22 nmol cm-2 d-1) or so, contradictory to the text.

A: We have changed the figure accordingly and adjusted the text.

Line 315: sulfate flux seems to be lowest at B1 in August not in February and highest in February or April, contradictory to the text.
A: We assume that the reviewer refers to the difference in uptake calculated for the SO_4 gradient and the SRR for August 2012. We have clarified in the new Figure 4 that the sulfate flux data are the ^{35}S data.

Line 398/399 “: : :constraints decide on the result of this competition between these two processes.”
A: Done

3.) Technical Corrections
line 144: cut-off
corrected
line 147: replace “to force out” by “to push”
revised
line 149: “CH4 standards at 100 ppm and : : :”
inserted ’of’
line 156: (cm3) instead of (cubic centimeter)
changed
line 162: missing dot after et al.
added
line 167: missing word after “adjacent”
inserted ’intervals’
line 177: 1 cm intervals

line 196: 40 L incubation tank

line 218: mL (“L” consistent to previous use)

changed
line 221: replace “to force out” by “to push”
changed
line 223: “CH4 standards at: : :”
’of’
line 227/228: 0.003 L / 0.009 L
inserted the space
line 257: 300 _M
µM

line 467/468: remove one “integrated” in the sentence.

Removed the first ’integrated’

Interactive comment on “Annual variability and regulation of methane and sulfate fluxes in Baltic Sea estuarine sediments” by Joanna E. Sawicka and Volker Brüchert

Anonymous Referee #2

Received and published: 23 August 2016

Review “Annual Variability and regulation of methane in sulphate fluxes in Baltic Sea estuarine sediments.” by Joanna E. Sawicka and Volker Bruechert

Sawicka and Bruechert study the seasonality of methane flux and sulphate reduction in two coastal sites in Sweden. With estuaries being important players in the global methane cycle, it is important to gain more insight into the controlling factors of methane oxidation in these systems.

Major comments:
There are several assumptions in the manuscript that are not backed up by either data or references.
E.g.
Line 47-48: Importance of advective processes.

See comments below

Line 333-337: Temperature regulation inference.

A: Sole temperature regulation would imply that methane oxidation is less temperature-sensitive than methanogenesis preventing methane oxidizers from keeping up with the enhanced methane flux during summer. This requires significantly different values of Q_{10} of methanogens and methane oxidizers. Publications from lake environments and terrestrial environments, e.g., King (1992), Wik et al. (2014), Nguyen et al (2011) suggest that aerobic methane-oxidizing bacteria may have higher Q_{10} than methanogens, but this argument remains unproven for marine habitats. In case of anaerobic methane oxidation, it is difficult to argue for a temperature adaptation disadvantage of AOM compared to methanogenesis, because of the tight coupling between sulfate reduction and methane oxidation and the phylogenetic proximity w/ respect to 16S of ANME to known methanogens, but also with regard to Archaeal membrane lipid composition, which should be considered strong physiological regulator for cross-membrane transport.

Line 348 salinity variation.

A: Data shown in Table 1 indicate that the salinity for the different sampling periods varied little.

Line 480-483 Variability in methane concentrations not due to variability in methane oxidation rates <u>alone</u>

A: The emphasis here is on the word <u>alone</u>. The whole first paragraph of the discussion emphasizes the different regulatory processes that affect methane concentrations and two important ones are of course temperature and bottom water oxygen.

Line 490-492: Changes in the upward transport rate of methane

A: Methanogenesis rates can only increase due a temperature increase, since the availability of organic carbon for methane production in buried sediment does not change. These effects were modelled by Dale et al. (2006) and are discussed here.

Line 498-501. Migration of the methane saturation zone due hydrostatic pressure changes

A: There is acoustic echosounder evidence for free gas presence in these sediments and the authors have personal communcation (Tom Floden) evidence that the depth of the free gas zone as seen on the acoustic echosounder changes substantially from year to year. The mechanisms that affect gas migration in these sediment are manifold. They can have to do with atmospheric pressure changes, wind direction or affecting water levels and these in turn affect the solubility of methane at a given temperature. An additional parameter is groundwater movement. There is also geophysical evidence from other areas in the outer Himmerfjärden area that suggest groundwater seepage. This implies a complex aquifer hydrology that was not accessible with the coring methods used here, but that has indirect effects on methane solubility, advective transport, and effective methane flux. A mechanistic or model-based evaluation of all these processes is far beyond the scope of this paper and cannot be adequately addressed in addition to the data presented here. We have explained this in lines 547-553 in the text.

Line 519-520. The period of ice cover has low flux rates. Extrapolation of rates during open-water conditions for a whole year would therefore be overestimates.

A: Yes, we agree. To our knowledge, this is one of the very few studies that reports sulfate reduction rates in a fully ice-covered estuary. These rates were very low compared to the open-water season. Extrapolation of these rates for the ice-covered period will necessarily give lower annually integrated rates compared to rates based on open-water measurements in spring/summer/fall. During certain years, the ice-covered season lasts from late December to early April. Ignoring winter data for extrapolations would give very erroneous numbers.

The authors go back and forth about stating if the methane transport is controlled by diffusion or advection. Sediment permeability would help to understand what role advection can play.

A: We do not have measurements of permeability available. While this could help, we would like to point out that even low-permeability sediment emit bubbles.

They state that changes in the hydrostatic pressure drives the changes in methane profiles, but do not explain what drives the changes in hydrostatic pressure, nor if that is related to season or not.

A: Changes in hydrostatic pressure in the Baltic Sea are influenced by air pressure, prevailing wind direction, and general sealevel stand due to the balancing effects of saltwater entry through the Danish straits and freshwater discharge in the northern Baltic, and from the rivers flowing in from the south. Additional effects are caused by the local coastal topography. These multiple parameters result in complex subsurface hydrology and complexity in estuarine water level conditions that make it difficult to use general meteorological observations to predict local sealevel variability. Hydrographic data are only reported for the general area in open water at the Landsort island, but these are not the same as in the archipelago.

A lot of equations are listed that are just taken from other publications. Those do not need to be listed again.

A: We have chosen to retain the formulas. There are enough readers that are unfamiliar with some of the methods. Without going into too much detail, these equations provide the basic framework.

It helps reading the manuscript if you keep the order of things the same, best throughout the manuscript (e.g. first mention station B1 then H6) but definitely in the same or consecutive sentences (e.g. Line 185-186, line 287 to 291). The order changes frequently in the manuscript making it harder to follow the arguments.

A: We have revised the manuscript to make sure the sequence is adhered to consistently.

Minor comments
Title "Annual variability : : :" if it is mostly the pressure it seems that the year plays not an important role here, so I think annual is not very good. If it is the seasons, I think seasonal is better. ": : : Baltic Sea estuarine: : :" but you say later that you investigated an estuarine and an open water station. Better say coastal?

A: Thanks for pointing this out. We changed this.

Abstract Line 41: You list 5.7mM as max in line 263 Line 43-4: ": : :lowering: : : far below the saturation concentrations." Your methane concentrations are also below the saturation concentrations below the sulphate penetration and seem to be mostly constant. Thus, the anaerobic methane oxidation does not seem to be lowering it far below the saturation concentration.

A: This is only observed for the winter observation and actually the major reason why we invoke an advective addition of methane. The other methane data are above saturation below the SMT.

Introduction Line 55: Would be good to put the Tg into perspective to the global flux to know the importance.

A: We have added data on the proportion of coastal emissions and compare them to the total estimated marine methane emission. However, the choice of a good number is difficult, partly because of the ongoing debate on the contribution of coastal methane emissions from thawing permafrost in the Siberian Arctic and uncertainties in correctly assessing macro/micro-seep-related emissions.

Line 66 and later in the methods: If the methane flux shows high spatial heterogeneity, why do you only measure the flux in one core?
The flux is the average of four core incubations and one diffusive flux measurement. Naturally, this variability is a sampling problem if porewaters are used.
Line 70-1 and 517-9: You also say you do not have data from the ice covered period (line 126)

A: This must be a misunderstanding, because we collected samples in late January/February with a ship capable of breaking the coastal ice.

Line 91: If you measure over four seasons, I feel seasonal is better than annual.

A: We changed this.

Materials and Methods: Line 102: Do you have info about the CH_4, POM and DOM of the effluent of the STP?

A: We have provided reported numbers on the treatment plant emissions for 2012. We would like to point out, however, that because of continuous improvement in the sewage treatment operation since the 80's, but at the same time the rapid growth of the Stockholm metropolitan area, the emissions and C/N/P composition have changed historically. Today's numbers may be misleading to understand the effects for buried sediment carbon from 20 to 30 years ago and only N and P data are available for the long period. For example, in 1994 the treatment plant treated the sewage of 250000 people for the southern Stockholm area. Today, the plant operates at its capacity and treats the sewage of 314000 people + 35000 additional people equivalents from industry (http://www.syvab.se/himmerfjardsverket/energi-och-materialflode), but N and P emissions are at pre-1970's levels.

Line 113: How thick is the rusty brown surface layer?

A: It changes significantly from 1 cm to complete absence.

Line 113-116: Do you have information about the grain size or permeability? That would really be needed to argue for or against advective transport.

A: These sediments are fine-grain muds with a small sand fraction. No exact grain size has been performed.

Line 125-6: What did it mean that there was ice coverage? Line 127:"until for the experiment" change Line 133: "1N HCl" please change to 1M

A: Changed.

Line 134-5: Drying for 2 hours seems short.
Did you test if longer drying had an effect?

A: As a matter of fact, the actual period was longer than that since the samples were retained in the oven. The calculated porosities are consistent with measurement from nearby stations.

Line 139: "seconds" seems a bit overstated, especially knowing that it takes already probably more than seconds before the core was on the ship.
"less that a minute" still sounds very impressive and is more realistic.

A: Less than a minute passes before we start removing samples. To avoid doubt, we have changed this to minutes since the whole procedure takes about 5 minutes for a Multicore.

Line 144: "exactly" delete

A: Changed

Line 145: "5M NaCl" that is not a standard treatment, did you test if it halted microbial activity? Why did you not use base?

A: If one calculates the solubility of CH_4 in 5M NaCl, CH_4 solubility is negligible, and due to the osmotic effect microbial activity likely ends very quickly as well. We do not agree that this is a non-standard method. There are plenty of publications, which use strong NaCl brine. NaOH is useful, if one is interested in the DIC concentration, but in our case we did not process for DIC. Also, the resultant solution is harmless and

cheap (kitchen salt) as opposed to strong base. In addition, the exchange of clay-bound CH_4 is enhanced in strong salts, which cannot be achieved with 2.5% NaOH.

Line 146: If you only leave the sample for 1 hour you will not get all the gas adsorbed to clay minerals.

A: This is very likely the case in these muddy samples.

Did you do later measurements to determine if the concentrations were constant?

A: Yes, the concentrations remain constant. We have conducted long-term tests, but for the sake of space in the methods description, we chose not to include every aspect.

Line 149: What column did you use on your GC?

A: The GC is equipped with a Porapak Q pre-column (3 feet) followed by a Hayesep D (9 feet) analytical column. We use two sequential 1ml+1ml loops for injection with luerlocked glass syringe. Injection occurs through a dried Na_2SO_4 filter bed with quartz wool endings. Flow rate of the GC is 60 ml flow rate (N_2 5.0). The FID operation occurs with zero air produced clean over a carbon filter and H_2 is produced with a Schmidlin H_2 generator. Gas cleanup occurs via activated carbon-cleaned cartridges. More details have been added to the method description.

Line 162: “10% HCl” is that 10% concentrated HCl in water or is that a 3.7 dilution of concentrated (37%)

A: We changed this to 1 M HCl.

Better give M concentrations.

Line 174: Why did you add cold sulphate to the tracer solution? That introduces sulphate into the sulphate free zone and does not do much in the not sulphate free zone.

A: We disagree. Non-amended tracer yields unrealistically high rates in the SMT because of limited sulfate and very high tracer turnover up to 50% of he added tracer, i.e., this would not be a tracer experiment any longer. In order for all incubations to be considered equivalent, a tracer turnover of less than 1% during the incubation is desirable to avoid kinetic limitation due to Michaelis Menten effects, irreversibility effects, etc. An additional beneficial effect is that potential SRR below the SMT can be detected and a cryptic sulfur cycle can be recognized.

Line 185: “(SO42-)”does not appear in the formula

A: Thanks. We corrected this.

Line 188-189: If you only show the median, what is the error? Plot it in the graph, or if it pretty much constant, state it here.

A: The standard error is now reported in the revised Figure 4. Replicate measurements are shown in Figure 3.

Line 215: Why do you use a different fixing agent for the methane samples? Why do you not use base?

A: See above.

Line 225-229: The list of variables and the equation do not fit to each other.

A: We adjusted the units.

Line 233-4: Volumetric units do not match.

A: Thanks: We changed ppm to nmol.

Line 237-42: Did you see any signs of burrows in your cores? Why do you only do one replicate if you know that it is spatially heterogeneous?

A: Marenzelleria is the dominant bioturbator in these sediments, but does not play a large role for CH4 emissions (Bonaglia et al., 2013 MEPS). Microelectrode profiling (Bonaglia et al., 2014) has not shown distinct subsurface increase due to pumped O_2 from burrowing or bioirrigating animals. Bioturbation is seen at Station B1 in the topmost 2 cm as described in the text. These, however, do not pertain to the diffusive flux calculations done for the SMT processes. As far as accounting for the degree of bioturbation is concerned, the resolution of the sampling at the sediment surface is the same as the bioturbation depth. Therefore, there is no possibility to account for bioturbation at the resolution of the sediment sampling.

A: The whole-core incubations are based on core replicates, the methane porewaters are singular cores. Our choice of data is a balance between replication, station time, sample numbers. Almost all core studies on porewater methane in the literature are on singular cores. We have conducted selected replicate experiments, but not for the whole dataset.

Line 248: “Do” why was it recalculated?
A: D^o is sensitive to temperature and salinity. See Boudreau (1996).

Results
Line 263: In the abstract you state that methane concentrations exceeded 6mM.

A: We corrected to 5.7 mM.

Line 267-270: it is hard to see linearity or concave shapes in log plots. If these are important don’t use log plots.

A: We have followed the recommendations by the reviewers, and have changed to a linear scale in addition to showing sulfate and methane data in the same graph (Figure 2a-h). The decision in favor of logarithmic plots was primarily because we wanted show the variability of the pore water concentration relative to the saturation concentrations during the different observation periods. In addition, the range of the concentration changes in a core is very substantial so that a logarithmic scale does better justice to the variability within a core (see ODP/IODP results). The in–situ saturation limit lines disappear if the natural range of concentrations were shown.

Line 271: You state that the methane profiles cannot be explained by the T and Corg changes. How about the sulphate profiles?

A: Our analysis indicates that the gradient of sulfate is as much influenced by heterotrophic sulfate reduction as by methane oxidation. The two cannot be

separated, and it is therefore not possible to judge on T and org C changes for sulfate reduction alone.

Line 303: You would also get a rate in the sulphate free zone if you would have injected tracer only. If you use the sulphate concentrations from you profile to determine the sulphate reduction rate? It does not matter that the tracer is reduced. If there is no sulphate the rate is still 0.

A: We disagree. There are now a number of publications that demonstrate the existence of the cryptic sulfur cycle (Holmkvist et al., 2014 GCA), and also indications by e.g., Leloup et al (2009) Environmental Microbiology) that demonstrate the existence of active sulfate reducers below the SMT zone.

Line 305-7: Why is that happening?

A: An interpretation is provided in the text? See above.

Line 313-7: All those rates should not be negative. A negative oxygen uptake of the sediment means diffusion of oxygen out of the sediment. Did you test if you could detect changes in sulphate concentrations in the whole core incubations?

A: No, we did not check for changes in sulfate, since the sulfate concentration is large compared to the uptake and the precision of a sulfate analysis is no better than 100 µM. By convention, fluxes into the sediment are negative (i.e., oxygen and sulfate, whereas fluxes out are positive, i.e., CH_4. This is what is indicated in the table and text.

Line 319: how about reoxidation by oxygen not only iron? Why do you suddenly have reactive iron here, when you state in line 484 that there is no other electron acceptor available?

A: Bonaglia et al (2014) give oxygen penetration depths for these sites. These are between 100µm and 0.5 cm. If there is a lack of a sulfate gradient, it is more likely that iron is the intermediate oxidizing agent.

Line 319: You have to keep in mind that the methane profile does not really give you a rate of methane diffusion out of the sediment. If there is oxygen in the surface sediment there is likely aerobic methane oxidation as you discuss yourself. Thus, this is an over estimation of the methane flux, and you do not know by how much. You need to have methane concentration data at the scale of the oxygen consumption to determine that.

A: We agree. Therefore we have in addition conducted whole-core incubations, with the caveats that this method has due to depressurization effects. Published data often use porewater gradients and it is therefore useful to present both types of analyses.

Line 328-9: As your diffusion based fluxes are overestimated (see comment above) there is no agreement, and thus bioturbation and irrigation, as well as advection as a result of your stirring probably affects the flux.

A: The diffusion-based fluxes are not overestimated at depth, where the resolution is sufficient and oxygen plays no role.

Discussion
Line 334-7: Do you have data available to support that? Can you model that it does not fit?

A: We have added two sentences in lines 547-553 to explain what a satisfying model would need to deliver. Such a model would have to be rather advanced and is not available to us, and would probably not be sufficiently constrained by the necessary physical background data.

Line 342: How about rate studies in temperature gradient blocks, e.g. by Sagemann or Arnosti?

A: We have unpublished temperature gradient block data on SRR for Himmerfjärden sediment. These support the existence of a broadly psychrotolerant/mesophilic SRB community and support the statement in the text. Apart from that, we would like to point out that temperature gradient block experiments of the kind the reviewer refers to are not adequate to address the temperature hypothesis for seasonal changes.

Line 348: How big could the effect be with this difference in salinity?
A: It is not relevant, mostly because sulfur cycling in the topmost cm makes sulfate multiple times available far in excess of concentrations variations due to salinity changes.

Line 352: How much Corg comes from the sewage treatment plant?

A: Although considerable (1676 tons for 2012, now mentioned in the text), the sewage treatment emits relatively small amounts of POM and DOM compared to the inorganic nutrients nitrate and phosphate, which stimulate plankton production in the estuary (Bonaglia et al., 2014). It is the nutrient effect that is most relevant for the carbon cycle in the estuary, not the heterotrophic carbon. Carbon estimates of the contribution by the sewage treatment plant were done by Savage et al. 2010 and indicate a local effect surrounding the sewage treatment plant due rapid deposition in the near area surrounding the outflow.

How similar or different is the fjord thus to others?

A: The inner parts of the fjord share similarities with other eutrophied fjord systems, e.g., Oslo fjord, whereas the outer parts are quite pristine and may be comparable to other northern latitude fjord-type systems. However, many fjord systems bordering the Atlantic have significantly higher salinities so that sulfate reduction prevails over a thicker sediment layer than in these sediments. Another difference is the glacial and postglacial history of the Baltic Sea. During glacial times organic-poor lake sediments were deposited followed Fe-rich post-glacial clay that is also low in organic carbon. In other fjord sediments, this discontinuity from a freshwater to a brackish/marine phase may not exist. This will affect the methane generation potential and thereby the methane flux.

Line 357-9: Where do the high sedimentation rates come from if there is only low river runoff?

A: These sediments are accumulation bottom sediments, which have a significant proportion of resuspended fine-grained material that is transported laterally and deposited in the bathymetric depressions of the fjärd.

Line 385: Table 1 has no information about the burial of organic material. Do you have depth profiles supporting that?

A: Published organic carbon concentration profiles can be found in Thang et al. (2013). Mass accumulation rates of organic carbon are reported in Thang et al. to be 9

– 9.5 mol m^{-2} y^{-1} (24 - 26 mmol m^{-2} d^{-1}) for a station close to H6. At Station B1, we do not have information of mass accumulation. However, data for similar sediments suggest C_{org} MAR of 3.3 mol m^{-2} y^{-1}. We have added these data to the site description section.

Line 391: Salinity of B1 is 7‰.

Line 392-4: A little too often “compar*”

We have modified the text to address unnecessary repetitions.

Line 392-8:
Lower sulphate concentrations mean that there is less sulphate available for organoclastic & methanotrophic sulphate reduction, just by simple numbers.

We do not dispute this. This is stated in lines 428-432.

Line 410&2: Figure 4

Line 412-3: Iron and manganese reducers do not always outcompete sulphate reducers, see work by Thamdrup and Vandieken.

Yes, but in these sediments this is the case. We have conducted bag incubation and iron and Mn speciation analysis in bag incubations in core profiles to 10 cm depth. These data indicate that BSR account for 75% of anaerobic organic matter oxidation, Fe reduction about 6.5 % and Mn reduction about 2.5%. The rest is accounted for by heterotrophic denitrification (Goldschmidt presentation Downs and Bruchert (2013); Bonaglia et al. (2014) Biogeochemistry).

Line 414-5: You state that the main driver for the differences is the advective flow based on the hydrostatic pressure, but here you speculate about more sulphate reduction leads to less methanogenesis. Which process is now the important one?

A: W would like to emphasize that it is not one OR the other, but an interplay of multiple processes with varying influences on the system. This is also why these sediments would be extremely hard to model accurately in one-dimensional reaction-transport models.

Line 417-20: How deep is the bioturbation in these sediments?
A: An exact bioturbation depth would be arbitrary. Macrofauna analysis at H6 and B1 has shown that Marenzelleria does generally not go deeper than about 4 cm, but can occur occasionally down to 10 cm.

Line 419-21: In advective systems with bioturbation fluxes should increase not decrease.

A: We disagree. Since more oxygen can be imported, it is possible that methane oxidation increases.

Line 443-5: This is not an explanation, it is just stating that you believe the data in contrast to the scenario below.

A: Possible explanations are provided in the following lines. 479-487

Line 454: “law” replace with “function”
A: Done

Line 461-3: Sentence not clear

A: We rephrased the sentence: now lines 498-500

Line 465: What is the percentage if you compare the methane flux into the SMTZ with the accumulated SRR or the total methane flux with the SRR? Do the numbers fit what model says?

A: Lines 503 state what the reviewer asks for, i.e., the depth-integrated SRR relative to methane flux rates fit well with the model.

Line 474-7: In line 445 you state that there is only little link.

I am sorry but we do not see the apparent contradiction the reviewer states.

Line 480-3: Please provide some support for this, maybe with a model.

A: The data showed an abrupt decrease in porewater concentrations from Oct 2012 to January/February 2013 for which an explanation is required. The above discussion intended to lay out that neither temperature, salinity, nor changes in organic matter influx alone can explain this change. The sentence does not intend to say more than that. It is beyond the scope of the paper to develop a unifying model that can address these processes satisfactorily. Even some of the currently most complete models, such as by Mogollon et al. (2011) JGR are idealizations that may not yield satisfactory fits with our data, but that does not necessarily dispute either model or data.

Line 484: Did you determine the concentrations of other electron acceptors like Fe? In line 319 you state that it is available for sulphide reoxidation.

A: Fe data are available from the nearby Station H5 and published in Thang et al. (2013). In addition, there are an unpublished data for nearby Stations H3 and H2 that indicate the limitation of reactive iron in the postglacial mud. In the glacial lake clays, however, reactive iron is more abundant again, but these latter sediments do not control the methane production, because their C_{org} contents are too low.

Line 490-2: Do you have any data on changing pressures? What would drive these changes? What would the possible magnitude be?

We have tried to obtain water level data and air pressure data for the periods of observation at the sampling stations, but these were not archived or could be found for the precise localities, only for the open Baltic nearby. However, local data is what was needed to have an accurate idea of the hydrostatic pressure.
Line 500-1: Do you have any data or reference to support this magnitude?
The best reference to address this question is the study by Mogollon et al (2011 JGR Biogeosciences), who modelled the free gas depth and AOM rates for two stations in southwestern Baltic Sea sediment. In that study temperature, and not tidally influenced pressure change, were found to be the dominant regulators of the free gas depth variation. Our differing intepretation is based on the observation that the seasonal variability in temperature at the two stites studied there are much greater than the ones studied here.

Line 500: “may as much as: : :” insert “be”

Done
Line 497-503: If it is the hydrostatic pressure it is actually not really a seasonal effect?
Yes
Conclusion
Line 508-9: Sentence not clear.

Line 515-6: How is it seasonal/annual if it is the hydrostatic pressure? Not clear.

Line 517-9: You also state that you do not have data from the ice covered times!
This must be a misunderstanding, since we have the February data. This is one of the few study that present data for conditions during ice cover.
Line 519-20: Why is that if the temperature and Corg input do not play an important role?
We emphasize that there are two aspects to be considered. Our winter and early spring data give low rates. Generally, in the literature, there are very few data available for sites with measurements during ice cover. If late spring/summer/fall rates are therefore extrapolated over a whole year, overestimates can result. Models, however, may account for this effect, if they are able to parameterize temperature and reactive organic carbon correctly (and the hydrological complexities of an archipelago setting riddled with fault lineaments). Secondly, while our observations indicate that hydrostatic changes or changes in porewater advection may have a considerable influence, this still does not take away from the fact that there is also seasonal variability. To model the annual variability based on organic carbon and temperature alone may therefore unfortunately also give the wrong results, because these factors have not been acounted to a degree that may reflect the specific regional situation. These two aspects need to be considered separately.

Line 520-1: Is it no advective or diffusive transport that controls the methane? You keep changing your argument.
Please see the comment above.

Tables
Table 2: “no AOM zone3” for H6 January 2013, change to “no AOM zone4”

Table 3: “Exponential coefficient (a)” this one is not exponential.
Will correct this
Figures Make all the y axis for depth the same scale.
Will correct this.
Figure 1. The colors do not help reading the map too much, not very clear in black and white at all. Change continent to just white or black to make ocean more clear and maybe reduce shades in the water.
We will consider a black and white map, or a map with sufficient contrast, clear legends and geographic locations.
Figure 2: You use the maximum sulphate penetration from figure 3 here. Maybe change the order of the figure to keep the flow consistent.
We will do that and then consequently also change the sequence in the results description.
Figure 3: How do you define your maximum sulphate penetration if there is a sulphate peak in your graphs below it?
Sulfate penetration depth was defined as the first lowest concentration measured. It is a common observation that traces of sulfate in the range of concentrations 50µM to 400 µM remain detectable in porewaters to substantial depths of several meters far into the methanogenic zone. This observation has been addressed in detail by Roey et al., Holmkvist et al and is part of the argument in favour of a cryptic sulfur cycle, which the authors of this manuscript also agree to. We also observe non-zero sulfate concentrations in the methane-rich zones of our sediment cores. We have therefore

chosen to define the sulfate penetration depth as that at which the sulfate changes sharply and the sulfate concentration is below 0.5 mM. We think that this combined criterion best reflects the condition of a transition from a dominating sulfate reduction environment to an environment in which methane-cycling processes start to prevail (while acknowledging low rates of cyptic bacterial sulfate reduction).

Figure 5: Keep order consistent between listing in the graph and in the caption. If you keep all the values positive it is much easier to compare the values. Which method are the methane fluxes based on?

We use the convention from the perspective of the water column, i.e., loss fluxes are fluxes into the sediment and negative and gain fluxes into the water column are positive.

Annual variability and regulation of methane and sulfate fluxes in Baltic Sea estuarine sediments

Joanna E. Sawicka and Volker Brüchert

Department of Geological Sciences, Stockholm University, Stockholm, 10691, Sweden

Correspondence to: Volker Brüchert (volker.bruchert@geo.su.se)

Abstract. ~~The effects of~~Marine methane emissions originate ~~for the most part~~largely from near-shore coastal systems, but ~~the~~ emission estimates are ~~generally~~often not based on temporally well-resolved data or sufficient understanding of the variability of methane consumption and production processes in the underlying sediment. The objectives of our investigation were to ~~investigate~~explore the effects of seasonal temperature, changes in benthic oxygen concentration, and historical eutrophication on sediment methane concentrations and benthic fluxes ~~were investigated~~ at two type~~-~~ localities for open-water coastal and eutrophic, estuarine sediment in the Baltic Sea. Benthic fluxes of methane and oxygen, sediment porewater concentrations of dissolved sulfate, methane, and ^{35}S-sulfate reduction rates were obtained over a 12-month period from April 2012 to April 2013. Benthic methane fluxes varied by factors of 5 and 12 at the offshore coastal site and the eutrophic estuarine station, respectively, ranging from 0.1 mmol $\mathrm{m^{-2}d^{-1}}$ in winter at an open coastal site to 2.6 mmol $\mathrm{m^{-2}d^{-1}}$ in late summer in the inner eutrophic estuary. Total oxygen uptake (TOU) and ^{35}S-sulfate reduction rates (SRR) correlated with methane fluxes showing low rates in the winter and high rates in the summer. The highest porewater methane concentrations also varied by factors of 6 and 10 over the sampling period with lowest values in the winter and highest values in late summer-early autumn. The highest porewater methane concentrations ~~exceeded 6~~were 5.7 mM a few centimeters below the sediment surface, but never exceeded the in-situ saturation concentration. 21 – 24% of the total sulfate reduction was coupled to anaerobic methane oxidation lowering methane concentrations below the sediment surface far below the saturation concentration. These data imply that bubble emission likely plays no or only a minor role for methane emissions in these sediments. The changes in porewater methane concentrations over the observation period are too large to be explained by temporal changes in methane formation and methane oxidation rates~~. Instead, it appears that advective methane recharge supplies of methane from deeper sediment layers to near-surface sediment. These are possible related to the transport of methane from deeper gas-rich areas or due to free gas movement or groundwater discharge~~ due to temperature alone. Additional factors such as regional and local hydrostatic pressure

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changes and coastal submarine groundwater flow may also affect the vertical and lateral transport of methane.

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Keywords **Methane cycling, coastal and estuarine sediment, seasonality** ~~Methane, sulfate reduction, estuary~~

1 Introduction

The world’s estuaries have been ~~estimated~~suggested to emit between 1.8 and 6.6 Tg CH_4 y^{-1} to the atmosphere (Borges and Abril, 2011; Amouroux et al 2002, Marty et al., 2001; Middelburg et al., 2002; Sansone et al., 1999; Upstill-Goddard et al., 2000~~).~~), a potentially considerable portion of the estimated total oceanic emissions of 10-30 Tg CH_4 y^{-1} (Judd, 2004; Etiope et al., 2008; Kirschke et al., 2013). As other globally upscaled estimates of emissions, these estimates also have considerable uncertainties. In the case of estuaries, a major cause of the uncertainty are relatively few spatially and temporally resolved measurements of anaerobic carbon degradation measurements in sediments and measurements of methane fluxes from sediments. In estuarine waters methane is produced by methanogenesis in underlying anoxic sediments, lateral freshwater or sewage discharge, seepage of methane-rich groundwater, or transport in ~~the~~the near-shore by aquatic plants (Borges and Abril, 2011). The amount of sedimentary methane production in estuaries is a function of organic matter availability, bottom water oxygen concentrations, and the salinity of the estuary. Methane production is generally greater in low-salinity estuaries because of lower sulfate availability to promote bacterial sulfate reduction (Borges and Abril, 2011). Methane fluxes from estuarine sediments are characterized by significant spatial and temporal variability (Borges and Abril 2011). Temporal patterns show that concentrations and fluxes of CH_4 are generally higher in the warmer ~~summer~~ season and low in the colder season (Crill et al., 1983, Martens and Klump, 1984, Musenze et al., 2014; Reindl and Bolałek, 2014). Notably, very few studies have considered CH_4 fluxes in high-latitude environments during snow- and ice-covered periods. While shallow systems within the tidal range derive a significant amount of the methane flux from ebullition (Martens and Klump, 1984), groundwater discharge, tidal pumping, and transport by aquatic plants (Middelburg et al., 2002; Kristensen et al 2008), the transport from deeper systems such as fjords and fjärds is thought to occur largely by molecular diffusion (Abril and Iversen, 2002, Sansone et al., 1998).

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Globally more than 90% of methane produced in marine sediments is estimated to be oxidized by the anaerobic oxidation of methane (AOM), mostly in the sulfate-methane transition zone (Knittel and Boetius, 2009, Martens and Berner, 1974; Jørgensen and Parkes, 2010). It is not known how much methane is oxidized by AOM in estuarine sediments. In addition, up to 90% of the remaining methane that reaches the sediment surface may be oxidized aerobically at the sediment surface or in the water column (Reeburgh, 2007). Yet, methane concentrations in estuarine waters are almost always higher than the atmospheric equilibrium concentration indicating that microbial oxidation processes and physical exchange with the atmosphere in estuaries are relatively inefficient in removing methane.

Despite its obvious importance, only few studies have specifically addressed anaerobic oxidation of methane by sulfate and aerobic oxidation in estuarine environments (e.g., Treude et al., 2005, Thang et al., 2013).

The ~~aim~~objective of this study was therefore to further elucidate mechanisms behind temporal variability of methane fluxes in a high-latitude coastal and estuarine environment with strong seasonal temperature variability, winter ice cover, and variable degree of eutrophication stress. These data fill an important gap of global inventories of nearshore sediment methane dynamics and help improve our mechanistic understanding of methane emissions from marine near-shore systems. We determined porewater concentrations of methane and sulfate, measured sulfate reduction rates with the ^{35}S-sulfate tracer method, and conducted core incubations to determine benthic fluxes of methane and oxygen at two deep stations of a low-salinity Baltic Sea estuary inside and at the opening of the estuary to the Baltic. Investigations were carried out over four seasons to capture the annual variability of chemical and biological conditions at the sediment surface and their influence on methane dynamics.

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2 Materials and methods

2.1 Site description

Himmerfjärden (Figure 1) is a fjord-type estuary with a surface area of 174 km^2 and a N-S salinity gradient increasing from 5.5‰ in the inner part to 7.0‰ at the opening to the Baltic. It is morphologically characterized by four basins, divided by sills~~. Water discharge to the estuary is ~~ and has a low ~~(~~flushing rate (~0.025/day) ~~and derives from~~(Savage and Elmgren, 2010). In 2012, the freshwater discharge comprised land run-off and precipitation (~~33~~ 30% and ~~14~~21% respectively), outflow from Lake Mälaren in the north (~~46%),~~19%) and the river Trosaån (23%), and discharge ~~of~~from a ~~major~~ sewage treatment plant (~~STP) (7%) (Boesch~~6%) (Larsson et al., ~~2006; Engqvist, 1996).~~2012). The ~~STP~~sewage treatment plant, built in the early 1970s, treats sewage water from ~~300~~ca. 314,000 inhabitants of the southern Stockholm metropolitan area, and its inorganic effluent is discharged mainly in the form of inorganic nitrogen and phosphorus to the inner basins (Savage and Elmgren, 2010). In 2012, the sewage treatment contributed 45% of the total phosphorus and 57% of the total inorganic nitrogen discharge to the northern Himmerfjärden area (Larsson et al., 2012) and discharged 1676 tons carbon (measured as chemical oxygen demand COD) (Stridh, 2012). The estuary undergoes thermohaline stratification during late summer and autumn, especially in the inner part, which experiences regular seasonal bottom water hypoxia. The tidal range is low (few cm) and relatively cold bottom waters (1.5 - 9°C) dominate throughout the year. Water level can vary annually by about 50 cm depending on local wind and hydrographic conditions. Late-summer-early fall bottom water hypoxia has also been reported occasionally for the outer basins of the estuary when winds are weak and circulation is inhibited (Elmgren and Larsson, 1997). Sedimentation areas in Himmerfjärden can be divided into accumulation and transport bottoms (Jonsson et al., 2003). About 21% of the sediment surface in Himmerfjärden is classified as accumulation bottoms of particulate material and receives 3.3-9 mol C m^{-2} y^{-1} (Thang et al., 2013; Karlsson et al., 2010).

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Bottom water and sediment samples were taken from a station in the inner part of Himmerfjärden, Station H6, and from a station located outside the estuary, Station B1 (Figure 1). Samples were collected in April, August, October 2012, and in February 2013. In addition, in April 2013 whole-core incubations were performed to determine methane and oxygen fluxes to record a full year of seasonal variability. Station B1 has soft, olive grey, muddy sediment with a 1-2 cm-thick rusty brown surface layer, while the sediment at station H6 is soft, laminated black mud with a 1-2 mm thin brown surface layer that ~~occurs~~occurred only during the winter and spring. Sediment accumulation rates range from 0.98 cm yr^{-1} in the innermost part of the estuary to 0.77 cm yr^{-1} in the outer part of the estuary (Thang et al., 2013).

2.2 Sample collection

Sediments with well-preserved sediment surfaces were collected with a Multicorer in acrylic tubes (9.5 cm diameter) to 40 cm depth to determine ^{35}S-sulfate reduction rates, porosity, and the porewater constituents methane and sulfate. Additional cores were collected for sediment core incubations. Porewater methane samples were immediately collected on-board from the cores as described below. The other cores were capped with rubber stoppers, transported to the marine laboratory on the island of Askö within 90 minutes and kept cold at bottom water temperatures for later experiments and subsampling. In February 2013, ice partially covered Station B1 and there was ~~full~~complete ice ~~coverage~~cover at Station H6, and sampling was only possible after ice breaking. For whole-core incubations, 30 l of bottom water was collected with a 5 liter HydroBios bottle and kept cold until for the experiments. Temperature, salinity, and oxygen concentrations were determined with a handheld WTW Oxygen meter directly in the water overlying the sediment cores.

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2.3 Organic carbon concentrations and porosity

Surface sediment concentrations of organic carbon were determined on freeze-dried sediment with a Fisons CHN elemental analyzer after treatment of freeze-dried sediment with ~~1N~~1M HCl to remove inorganic carbon. Water content (%) was determined by drying 5 ml of sediment at 105°C for two hours and calculating the percent loss after drying.

2.4 Methane analysis

Samples for methane were collected directly through the side of taped, pre-drilled core liners and taken in 2-cm intervals ~~seconds~~minutes after the core was retrieved on deck. The core sampling method used in this study permits complete sampling and preservation of porewater methane within 5 minutes after the core was on deck. Under these circumstances, loss of methane due to gas loss is low and methane concentrations could be determined for porewaters that were far above the saturation limit at 1 atmosphere pressure for the salinity and temperature range of the bottom water (between 1.9 mM and 2.4 mM). A sediment sample of exactly 2.5 mL was taken with a 3 mL ~~cutoff~~cut-off syringe. The sample was transferred to a 20 mL serum vial containing 5 mL 5 M NaCl and immediately closed with a thick septum and an aluminum crimp seal. The sample was shaken~~, left for 1 hour for gas equilibration,~~ and 5 mL of brine was injected into a sample vial to ~~force out the~~displace 5 mL gas ~~samples~~ out of a vial into the syringe. The CH_4 measurements were carried out on a gas chromatograph (GC) with a flame ionization detector (FID) (SRI 8610C) ~~and~~after separation on a 3 feet Porapak Q pre-column before a 9 feet Hayesep D column with N_2 ~~was used~~ as carrier gas. CH_4 standards 100 ppm, 1000ppm, and 10000 ppm (Air Liquide) were used for calibration.

The concentration of methane (mM) ~~in the headspace~~ of a sample was calculated ~~from~~as follows:

$$CH_4(mM) = \cancel{\frac{V_{headspeace}A\alpha}{24.1V_{sediment}\rho}}\frac{CH_{4\ hsp}\cdot V_{hsp}}{1000\cdot 24.148\cdot V_{sed}\cdot \rho} \qquad (1)$$

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where ~~V_{head}~~$CH_{4_{hsp}}$ is the concentration of methane in the headspace of the sample vial (ppm), V_{hsp} is the volume of the headspace ~~in the sample vial (cm^3), ρ is the sediment porosity, A is the peak area of methane eluted, α is the slope of the standard curve (parts per million volume basis), and~~(L), V_{sed} is the volume of the sediment sample (~~cubic centimeter). The~~ L), ρ is sediment porosity, and 24.148 (L mol^{-1}) is the molar volume of ~~methane~~gas at ~~20 °C and 1 atm~~standard pressure ~~(24.148 L mol^{-1}) was used to convert from partial volume of CH_4 gas to the mole fraction of CH_4~~100 kPa and 298 K.

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2.5 Sulfate concentration

Porewater samples for sulfate concentration measurements were obtained using rhizones (Atlas Copco Welltech) (Seeberg-Elverfeldt et al 2005). Rhizones were treated for 2 hours in ~~10%~~2M HCl ~~solution~~, followed by two rinses with deionized water for 2 hours and final storage in deionized water. Rhizones were connected to 10 mL disposable plastic syringes via ~~a~~ 3-way ~~luer-type stop-cock~~luerlock stopcock and inserted in 1~~-~~-cm intervals through tight-fitting, pre-drilled holes in the liner of the sediment cores. The first mL of pore water was discarded from the syringe. No more than 2 ml were collected from each core to prevent cross-contamination of adjacent ~~due to the porewater suction~~intervals (Seeberg-Elverfeldt et al., 2005). Sulfate ~~concentration was measured on~~concentrations were determined with a Dionex System IC 20 ion chromatograph.

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2.6 ^{35}S-Sulfate reduction rates

To determine bacterial sulfate reduction rates (SRR) sediment cores were subsampled in 40-cm long 28 mm-diameter cores with 1-cm spaced, silicon-sealed, pre-drilled small holes on the side for injections. For the incubation, the whole-core incubation method by Jørgensen (1978) was used. $^{35}SO_4^{2-}$ tracer solution was diluted in a 6 ‰ NaCl solution containing 0.5 mM SO_4^{2-} and 2.5 µl of the tracer solution (50kBq) was injected through the pre-drilled holes. The cores were then capped and sealed in plastic wrap foil and incubated for 8 hours at the respective bottom water temperatures. After this time, the incubations were stopped by sectioning the core in 1-cm intervals to 5 cm depth and in two centimeter intervals below this depth to the bottom of the core. Sediment sections were transferred into 50 ml plastic centrifuge tubes containing 20 ml zinc acetate (20% v/v) and shaken vigorously and frozen. The total amount of ^{35}S-labeled reduced ~~inorganic~~ sulfur (~~TRS~~TRIS) was determined using the single-step cold distillation method by Kallmeyer et al. (2004). ~~TRS~~TRIS and supernatant sulfate were counted on a TriCarb 2095 Perkin Elmer scintillation counter. The sulfate reduction rate was calculated using the following equation (Jørgensen, 1978):

~~$^{35}SRR = \frac{TRI^{35}S\ \rho\ 1.06}{(^{35}SO_4^{2-} + TRI^{35}S)\ T}$ (2)~~

$$^{35}SRR = \left(TRI^{35}S \Big/ (^{35}SO_4^{2-} + TRI^{35}S) \right) \cdot 1.06 \cdot SO_4^{2-} \cdot \rho \cdot 1/t \qquad (2)$$

where ~~(~~SO_4^{2-} ~~ρ)~~ is the pore water sulfate concentration corrected for porosity ρ, TRI^{35}S and $^{35}SO_4^{2-}$ are the measured counts (cpm) of ~~sulfate and~~ total reduced inorganic sulfur species and sulfate, respectively, 1.06 is a correction factor accounting for the isotope discrimination of ^{35}S against ^{32}S-sulfate, and ~~T~~t is the incubation time. The sulfate reduction rate is reported as nmol cm^{-3} day^{-1}. Generally, when cores were available ^{35}SRR were measured ~~in three parallels~~on replicate cores for all depth intervals ~~and the values reported here are the median values of the triplicates~~. The detection limit of the rate measurements accounting for distillation blanks and radioactive decay of ^{35}S between experiment and laboratory workup was 0.1 nmol day^{-1} cm^{-3}.

2.7 Whole-core sediment incubations

Four intact cores with undisturbed sediment surfaces and clear overlying water were subsampled in the laboratory in acrylic tubes (i.d. 6.2 cm, height 25 cm) retaining about 10 cm of the overlying water. The sediment height in the tubes was approximately 10 cm. The cores were incubated in a 40-liter

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incubation tank filled with bottom water from the same station. Before the incubation the overlying water in the cores was equilibrated with bottom water in the tank. The overlying water in the cores was stirred by small magnetic bars mounted in the core liners and driven by an external magnet at 60 rpm. The cores were pre-incubated uncapped for 6 hours and subsequently capped and incubated for a period of 6 to 12 hours depending on the initial oxygen concentration in the bottom water.

2.8 Total oxygen uptake

Oxygen sensor spots (Firesting oxygen optode, PyroScience GmbH, Germany) with a sensing surface of a diameter of 5 mm were attached to the inner wall of two incubation cores (diameter 5.5 cm). The sensor spots were calibrated against O_2-saturated bottom water and oxygen-free water following the manufacturer's guidelines accounting for temperature and salinity of the incubation water. Measurements were performed with a fiberoptic cable connected to a spot adapter fixed at the outer core liner wall at the spot position. The O_2 concentration was continuously logged during incubations. Sediment total oxygen uptake (TOU) rates were computed by linear regression of the O_2 concentration over time.

2.9 Methane fluxes

Methane fluxes were determined from discrete water samples collected in 12 mL Exetainers (Labco, Wycombe, UK) prefilled with 50 µL of 50% $ZnCl_2$ without headspace. Samples were collected at the beginning (time zero) and at the end of the incubation (time final), usually after 24 hours. CH_4 concentrations were determined using the headspace equilibration technique (Kampbell et al., 1989) by ~~displacing~~replacing 3 ml of the water in the exetainers with high-purity helium gas at atmospheric pressure. The Exetainers were then shaken at 400rpm on a shaking table for 60 minutes to allow the gas to equilibrate between the headspace and the liquid phase and left to rest for half an hour. After equilibration 2.5 mL of NaCl brine was injected into an Exetainer to force the gas samples into an injection syringe while maintaining the headspace pressure. The samples were injected onto a 1 ml injection loop of a gas chromatograph (SRI ~~8610~~8610C) with FID detector using N_2 as carrier gas. CH_4 standards 5 ppm, 100 ppm and ~~10000~~1000 ppm (Air Liquide) were used to construct a calibration curve.

~~Partial~~The partial pressure of CH_4 in the equilibrated headspace and water was calculated using the solubility coefficient β for CH_4 (Wilhelm et al 1977), gas constant R (8.314 L kPa mol^{-1} K^{-1}), air pressure ~~(~~P ~~in (~~kPa), headspace gas concentration $CH_{4\,\cancel{(}hsp\cancel{)}}$ ~~(ppm~~ (nmol), headspace volume (0.003L), water volume in the exetainer (0.009L), and laboratory temperature T (293 K) according to

$$CH_4\ (nM) = (CH_{4\,\cancel{(}hsp\cancel{)}} + \beta\, CH_{4\ hsp}) * P/RT \qquad (3)$$

Fluxes (J) of CH_4 (mmol m^{-2} d^{-1}) during the whole core sediment incubations were calculated according to

$$J = (CH_{4\ start} - CH_{4\ end})/\cancel{T}t * V/A \qquad (4)$$

where $CH_{4\ start}$ and $CH_{4\ final}$ represent the end and start concentrations in mmol/m^3, V is headspace volume (~~L~~m^3), A is the surface area of the incubation core (m^2), and ~~T~~t is the incubation time (days).

2.10 Diffusive flux calculations

Diffusive fluxes of methane and sulfate were estimated from the porewater gradients of methane and sulfate for the sediment surface and the sulfate-methane transition zone. Sediment cores at station B1 showed occasional burrows from deposit feeders in the topmost 2 cm of sediment, whereas sediment at station H6 was largely devoid of macro- and meiofauna. Since only one sample was taken from the topmost 2 cm, quantitative depth-related effects of bioturbation cannot be accounted for in this analysis

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and upward diffusive transport of methane was assumed as the dominant transport pathway. Fluxes were estimated using Fick’s first law of diffusion

$$J = D_s \frac{dC}{dx} \qquad (5)$$

assuming that flux was dominated by molecular diffusion, where dC is the change in concentration of dissolved sulfate (mM) or methane (mM) over a depth interval dx (cm), and D_s is the sediment diffusion coefficient corrected for temperature and salinity according to Boudreau (1996). D_s was recalculated from the molecular diffusion coefficient D_o for sulfate and methane according to Iversen and Jørgensen (1994).

3 Results

3.1 Bottom water temperature, dissolved oxygen, organic carbon

~~During~~Over the observation period April 2012 through February 2013 bottom water salinity varied between 6.5 and 7.0‰ at station B1 and 5.4 and 6.5‰ at Station H6 ~~and 6.5 and 7.0‰ at station B1~~ (Table 1), while bottom water temperatures ranged from 2.4°C to 6.9°C ~~at~~for station B1 and 1.8°C to 9.4°C ~~at~~for station H6. The lowest and highest bottom water oxygen concentrations ~~were~~ measured ~~in April 2012 (40 µM at station H6, and~~ were 160 µM ~~at~~for station B1~~)~~ and 40 µM for station H6 in April 2012, and ~~February 2013 (~~300 ~~at station H6~~ µM and 380 µM ~~at~~for station B1 and station H6 in February 2013, respectively~~).~~. Surface sediment organic carbon concentrations were similar at the two stations ranging between 4.6 and 5.2% at Station B1, and 5.0% and 6.0% at Station H6 over the observation period.

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3.2 Methane and sulfate concentrations

The highest methane concentrations in the sediment cores were recorded in August 2012, when they reached 1.9 mM at station B1 and 5.7 mM at station H6 ~~and 1.9 mM at station B1~~ (Figure 2a-h). Methane concentrations were lowest in February, when the highest concentrations in the cored

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sediment were ~~1.5 mM at station H6 and~~ only 0.1 mM at station B1 and 1.5 mM at station H6. The measured methane concentrations never exceeded the solubility limit for methane calculated for the *in situ* pressure, which ranged from 9.6 to 11.9 mM during the different sampling periods. Generally, methane concentrations at station H6 increased linearly from the surface down to 10 cm depth. Below this depth they only increased slightly or remained constant. An exception to this trend was observed in February at station B1, when the methane ~~showed a concave-upward trend indicating active~~concentration profile indicated net consumption of methane in the topmost 10 cm of sediment.

Sulfate concentration gradients changed considerably between the different seasons at both stations reflecting substantial changes in sulfate reduction rates over the observation period. At both stations, the sulfate concentration gradients were steepest in October, intermediate in April and August, and lowest in February indicating highest and lowest sulfate reduction rates in October and February, respectively (Figure ~~3 a-h).~~ 2 a-h). ~~At station H6, sulfate was always depleted in the cored sediment interval, albeit at substantially greater depth in February. Depletion already occurred at 5 cm depth in April and October and at 9 cm depth in August, and sulfate concentrations showed a typically concave downward gradient.~~ At station B1, sulfate was never consumed completely and concentrations remained above 1.5 mM at the bottom of the core. Generally, sulfate decreased steeply from the surface down to 10 cm depth in August and October. Below this surface zone there was an interval with nearly constant concentrations down to 20 cm depth, below which sulfate decreased again to a concentration to about 1.5 mM. Despite some variability in the sulfate concentration profiles and a lower gradient in the topmost centimeters in April and February, the sulfate concentrations at the bottom of the core were similar during all observation periods. At station H6, sulfate was always depleted in the cored sediment interval, albeit at substantially greater depth in February. Depletion already occurred at 5 cm depth in April and October and at 9 cm depth in August, and sulfate concentrations showed a typical concave gradient.

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3.3 ^{35}S-sulfate reduction rates

~~In agreement with the sulfate concentration gradients, ^{35}S-sulfate reduction rates were higher at station H6 than at station B1 (Figure 4 a-h). At station B1, SRR ranged from 0.2 nmol cm^{-3} d^{-1} to 63 nmol cm^{-3} d^{-1}, while at H6 SRR were as high as 411 nmol cm^{-3} d^{-1}.~~At Station B1, depth-integrated sulfate reduction rates over the core length varied from 0.5 to 2.3 mmol m^{2} d^{-1} and SRR ranged from 63 nmol cm^{-3} d^{-1} at the sediment surface to 0.2 nmol cm^{-3} d^{-1} at the bottom of the cored intervals (Figure 3 a-h, Table 2). The highest SRR were measured in the topmost 2 cm with the exception of October 2012, when the maximum was found at 3 cm depth. Below the maximum, rates decreased exponentially indicating that organoclastic sulfate reduction dominated and that the reactivity of the degrading organic material decreased exponentially with depth. Over the cored sediment interval, there was no second peak that could be attributed to significant AOM. Nevertheless, the distinct curvature of the methane concentration profile in February 2013 at station B1 indicates that methane was oxidized in the sulfate reduction zone and that some sulfate reduction was coupled to anaerobic methane oxidation. Organiclastic sulfate reduction and anaerobic methane oxidation co-occurred in these depth intervals. Overall, no clear seasonal trend was found in the rates and winter rates were comparable to summer and fall rates.

At Station H6, the highest measured SRR was 338 nmol cm^{-3} d^{-1} and occurred at 2 cm depth in April 2012. Organoclastic sulfate reduction dominated the interval down to 10 cm. Depth-integrated sulfate reduction rates over the core length varied ~~from~~ 9.2 to 11.7 mmol m^{-2} d^{-1} at station H6. In April, August, and ~~0.5 to 2.4 mmol m^{2} d^{-1} at station B1.~~ ~~Two~~October 2012 two distinct sulfate reduction rate peaks were found at station H6, one at the surface and a second peak between 10 cm and 15 cm depth. The latter is in the sulfate-methane transition zone and indicates that in this depth interval the rates of anaerobic methane oxidation coupled to sulfate reduction exceeded organoclastic sulfate reduction rates. ~~Depth~~Previous studies at nearby station H5 in Himmerfjärden also found AOM to be present at depths between 6 and 16 cm, which is in agreement with our findings (Wegener et al., 2012). The depth-integrated rates of ~~sulfate reduction~~ ^{35}SRR in the

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sulfate-methane transition zone at H6 were relatively constant over the three observation periods and varied between 2.4 mmol m^{-2} d^{-1} and 2.8 mmol m^{2} d^{-1} (Table 2). In February, however, when sulfate penetrated to 24 cm depth, sulfate reduction rates were about two times lower compared to the other months~~ and a second~~. The previously observed deeper sulfate reduction peak ~~coupled to methane oxidation~~between 10 and 15 cm depth was not visible~~.~~, although a SRR peak was observed between 5 and 9 cm depth. However, the ~~distinct upward concave curvature~~high concentrations of ~~the~~sulfate and low concentrations of methane ~~profile~~in this depth interval in February ~~at station B1 indicates~~make it unlikely that ~~even here some of sulfate reduction was coupled to anaerobic methane oxidation and~~ this peak is due to AOM. It is more likely that this ~~process overlapped~~peak is associated with ~~organoclastic~~organiclastic sulfate reduction, because no change in the sulfate or methane gradients was observed at this depth. Sulfate reduction was also detected below the sulfate-methane transition zone at station H6 in April, August, and October. Since non-radioactive carrier sulfate was added to the ^{35}S-tracer during these incubations, these rates indicate potential sulfate reduction activity in the methanogenic zone (Leloup et al., 2009). ~~The lack of the second peak in February at H6 is in agreement with previous observations that productive seasons lead to shoaling of the methane-dependent sulfate reduction activity and anaerobic oxidation methane layer in the sediments (Dale et al 2008, Treude et al 2005a). Previous studies at neighboring stations H2 and H3 found AOM present at the depths 6-16 cm and 16-28 respectively, which is in agreement with our findings (Wegener et al 2012).~~

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3.4 Benthic exchange of oxygen, sulfate, and methane

Rates of total oxygen uptake are summarized in Table 2 and shown for comparison in Figure 5. Total oxygen uptake was lowest in February at both stations (B1: -12~~±~~ ± 2.5 mmol m^{-2} d^{-1} and H6: -14.9~~±~~ ± 3.5 mmol m^{-2} d^{-1}~~)~~), and highest in April at station B1 (-33.5 ± 4.7 mmol m^{-2} d^{-1}) and in August at station H6 (-26.9~~±~~ ± 3.7 mmol m^{-2} d^{-1}~~) and in April at station B1 (-33.5±4.7 mmol m^{-2} d^{-1}~~). ~~The diffusive~~Diffusive fluxes of sulfate~~ fluxes~~ from the water column into the sediment ranged from -0.2 mmol m^{-2} d^{-1} in February to -1.4 mmol m^{-2} d^{-1} in October at station B1, and from -1.3 mmol m^{-2} d^{-1} in

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February to -2.7 mmol m^{-2} d^{-1} in August at station H6 (Table 2). These rates are significantly lower than the radiotracer rates and indicate that sulfate is reoxidized below the sediment surface by reaction with reactive iron (Thang et al., 2013). ~~Methane fluxes determined by whole-core incubation were consistently higher than the fluxes determined from the concentration profiles of dissolved methane at station H6, whereas the two methods gave similar results at Station B1 (Table 2). The seasonal variability in fluxes at the two stations was similar for the two measuring methods (Table 2).~~ Whole-core methane fluxes ranged from 0.1 mmol m^{-2} d^{-1} (February and April) to 1.2 mmol m^{-2} d^{-1} (August) at station B1 and from 10.3 mmol m^{-2} d^{-1} (February) to 19.9 mmol m^{-2} d^{-1} (August) at station H6~~, and from 0.1 (February and April) to 1.2 mmol m^{-2} d^{-1} (August) at station B1~~ (Figure 5, Table 2). ~~The very high value measured in August 2012 at Station H6 is likely due to ebullition during the incubation at ambient air pressure.~~ Diffusive methane fluxes ranged from 0.05 mmol m^{-2} d^{-1} to 1.6 mmol m^{-2} d^{-1} at Station B1 and from 0.4 to 2.6 mmol m^{-2} d^{-1} in August at H6. Methane fluxes determined by whole-core incubation and fluxes determined from the concentration profiles of dissolved methane gave similar results at Station B1, but whole-core incubation fluxes were consistently higher at station H6 (Table 2), but the general seasonal variability in fluxes at the two stations was similar with the two measuring methods (Table 2). The very high value measured in August 2012 at Station H6 is likely due to ebullition during the incubation at ambient air pressure. The good agreement between whole-core fluxes and diffusion-based fluxes at station B1 suggests that bioturbation and irrigation at this station had little influence on the methane exchange with the bottom water.

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4 Discussion

4.1 Bottom water temperature and salinity

Correlations between biogeochemical rates and fluxes with bottom water temperatures in Himmerfjärden between April 2012 and February 2013 were weak for the period April-October, and forced by the low rates in the coldest observation period in early February 2013. The temperature versus rate/flux relationships were generally non-linear and not consistent for the fluxes of oxygen, methane, and sulfate indicating that additional controlling factors played a role. It is likely that the microbial community involved in the cycling of methane and sulfur species in Himmerfjärden sediment is temperature-sensitive, and that the low rates in February 2013 are due to the 3°C temperature drop in bottom water from October 2012 to February 2013. This would be consistent with rate observations in comparable environments by Treude et al (2005a), Abril and Iversen (2002), Crill and Martens (1983), and Westrich and Berner (1988), and is also supported by studies of the microbial community composition of estuarine sediments that showed variations as a function of temperature (e.g., Zhang et al 2014). Regulation of methane fluxes largely by temperature implies that methane oxidation in Himmerfjärden sediment is less temperature-sensitive than methanogenesis preventing methane oxidizers from keeping up with the enhanced methane flux during summer. This requires significantly higher temperature stimulation of methanogens than methane oxidizers or lack of an electron acceptor for methane oxidation. Publications from lake environments and terrestrial environments suggest that aerobic methane-oxidizing bacteria may indeed be less temperature-sensitive than methanogens (King, 1992; Wik et al., 2014; Nguyen et al., 2011). However, this argument is not well supported for marine habitats. In case of anaerobic methane oxidation, it is difficult to argue for a physiological temperature disadvantage of methane oxidizers compared to methanogens, because of the tight coupling between sulfate reduction and methane oxidation, the phylogenetic proximity of ANME to known methanogenic Archaea (Knittel and Boetius, 2009), and similarities in membrane composition of ANME and methanogenic Archaea (Wegener et al., 2012). ~~HoweverRegulation of methane fluxes largely by temperature implies that methane oxidation in Himmerfjärden sediment is less temperature-sensitive than methanogenesis preventing methane oxidizers from keeping up with the enhanced methane flux during summer. This requires significantly higher temperature stimulation of methanogens than methane oxidizers or lack of an electron acceptor for methane oxidation. Publications from lake environments and terrestrial environments, e.g., King et al. (1988), Wik et al. (2016), Nguyen et al (2011) suggest that aerobic methane-oxidizing bacteria may indeed be less temperature-sensitive than~~

~~methanogens, but this argument is not well supported in marine habitats. In case of anaerobic methane oxidation, it is difficult to argue for a physiological temperature disadvantage of methane oxidizers compared to methanogenesis, because of the tight coupling between sulfate reduction and methane oxidation and the phylogenetic proximity of ANME to known methanogens (Knittel and Boetius, 2009; Wegener et al., 2012).~~ Further, microbial community composition and biogeochemical rates often cannot be directly established from binary relationships with temperature, since other physical and chemical parameters such as salinity, bottom water oxygen concentrations, organic carbon accumulation also vary seasonally. Of these, salinity is not considered to be important for the present study, because the annual range in Himmerfjärden bottom water was only between 5.4 and 7 ‰, which is too small to affect the major electron acceptor and carbon degradation pathways.

4.2 Effects of organic matter composition and sedimentation

Organic carbon concentrations in Himmerfjärden are comparable to other fjord- and fjärd-type estuarine sediments (Bianchi, 2007; Smith et al., 2015). Primary organic carbon export in Himmerfjärden varies strongly on both seasonal and interannual timescales. The major export periods occur during the spring phytoplankton bloom in March-April to early May, a late-summer cyanobacterial bloom in August, and a secondary phytoplankton bloom in September (Bianchi et al., 2002; Zakrisson et al., 2014; Harvey et al., 2015). Terrestrial-derived organic carbon that is not derived from the sewage treatment plant plays only a minor role in this system, because no major rivers enter the system and surface rainwater runoff is low. Based on sediment trap studies, the annual organic carbon flux in Himmerfjärden varies by more than an order of magnitude at station B1 and by about a factor of 3 in the inner parts of Himmerfjärden (Blomqvist and Larsson, 1994). However, only 10% to 60% of the total vertical mass flux may be composed of primary organic carbon, while the remainder has been interpreted as resuspended material (Blomqvist and Larsson, 1994).

A second effect to be considered is that stations B1 and H6 are located in bathymetric depressions. H6 is in the center of a sub-basin separated from the outer Himmerfjärd by a sill (Fig. 1). Likewise, Station

B1 is located in a small depression at the head of a submarine channel that opens to the Baltic Sea. Fine-grained and reworked organic-rich material preferentially accumulates in these depressions (Jonsson et al., 2003). Because of the importance of resuspended organic material for the vertical mass flux and bioturbation, the annual variability in the organic matter composition at the sediment surface varies year-round only between 5 and 6 % OC with relatively constant C/N ratios between 7.9 and 9.1 at Station B1 and 8.3 and 9.2 at Station H6 (Bonaglia et al., 2014). Organic mass accumulation rates in the accumulation bottoms based on ^{210}Pb dating are reported between 3.3 and 9.5 mol m^{-2} d^{-1} (Thang et al., 2013; Karlsson et al., 201~~3~~0). The combined effect of the sedimentation characteristics is that temporal variability in the ~~bottom~~ settling primary organic carbon flux above the sediment surface is low, which reduces the overall temporal variability in organic carbon amount and composition and thereby in carbon mineralization rates. This small temporal variability is further influenced by macrofauna bioturbation in the top 5 cm of sediment in this area, foremost by the bivalve *Macoma baltica*, the arthropod *Pontoporeia femorata*, and the polychaete *Marenzelleria* (Bonaglia et al., 2014). Although macrofauna is largely absent at Station H6, sediment is also mixed at station H6 by bioturbating meiofauna (mostly ostracods) (Bonaglia et al., 2014).

The measured benthic oxygen uptake rates are consistent with the low variability in the surface organic carbon concentrations, C/N ratios, and a temperature-dependent decrease in total oxygen uptake rates in winter. The slightly higher total oxygen uptake rate at Station H6 is also consistent with the physiography of the enclosed small basin favouring sediment trapping of fine material. In addition, the location of station H6 in the inner fjärd limits water exchange and leads to greater oxygen depletion, whereas the more open station B1 is affected by upwelling of oxygen-rich waters and comparatively less burial of organic material (Table 1).

4.3 Methane fluxes, sulfate reduction and methane oxidation

~~The~~Sediment focusing in the sub-basins of the inner Himmerfjärden sediments ~~have~~results in very high sedimentation rates between 0.9 and 1.3 cm/yr (Thang et al., 2013; Bianchi et al., 2002). In such sediments organic carbon burial and transfer of organic matter into the methanogenic zone is efficient and will occur within 20 to 30 years. As a consequence of the low bottom water salinity ~~(<~~of 6~~ ‰)~~‰ of the Baltic Sea at this latitude, seawater sulfate concentrations are less than 7 mM and, by comparison with normal seawater, a comparatively lesser amount of organic matter can be degraded by bacterial sulfate reduction (Thang et al., 2013). Consequently, compared to normal marine ~~sediment~~sediments a larger proportion of organic matter undergoes anaerobic microbial degradation terminating in methanogenesis, which generates a high upward flux of methane into the sulfate-containing zone. Organiclastic sulfate-reducing bacteria will compete for the available sulfate with sulfate-reducing bacteria involved in the anaerobic oxidation of methane (Dale et al., 2006; Jørgensen and Parkes, 2010). Thermodynamic and kinetic constraints decide on the outcome between these two competing processes. Dale et al. (2006) suggested that due to lower winter temperatures and greater sulfate availability in the sulfate-methane transition zone in winter, the thermodynamic driving force for anaerobic methane oxidation increases allowing for a greater proportion of anaerobic methane oxidation coupled to sulfate reduction in the winter. In the summer and fall, higher temperatures and sulfate limitation favor organiclastic sulfate reduction and methanogenesis while limiting the anaerobic oxidation of methane. Most importantly, however, their analysis showed that due to thermodynamic constraints and slow growth rates of the methane-oxidizing archaea the microbial biomass does not change significantly over a year. These conceptual modelling results can be tested with our Himmerfjärden data.

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Sulfate reduction rates, particularly at H6, demonstrate how strongly bottom-water oxygen controls organic matter mineralization. In the spring, summer, and fall sulfate reduction was at its maximum in the first two centimeters of the sediments (Fig 3 e, f, g). In February, reduced organic carbon input and higher oxygen concentrations resulted in lower sulfate reduction rates and a shift of the maximum rates to greater depths in the ~~sediments~~sediment (Figure 3 h). Since other terminal carbon-oxidizing processes (e.g. denitrification, iron, and manganese reduction) outcompete sulfate reduction for electron-donating substrates, the depth of sulfate penetration and organic matter degradation via sulfate shifts deeper in the sediment, which reduces methane production.

The decrease in oxygen uptake matches well with the decrease in methane fluxes at the two stations in winter, which suggests an impact of oxygen on methane cycling (Table 2, Figure 5). Higher oxygen levels enhance bioturbation and oxygen uptake by the abundant macro- and meiofauna (Norkko et al., 2015), but the mixing of sediment also affects methane transport to the water column, as the main transport process shifts from diffusion to advection. This effect is likely the main cause for the winter decrease in methane fluxes and concentrations. More aerated conditions indirectly enhance methane removal by sustaining aerobic methanotrophs (Valentine 2011). It is plausible that, as in other brackish coastal sediments, aerobic methanotrophs at the surface of Himmerfjärden sediments consume a significant part of upward-diffusing methane that was not oxidized by anaerobic methane oxidation (McDonald et al 2005, Moussard et al 2009, Treude et al 2005a).

Published benthic methane fluxes for estuaries with similar salinities have a reported range of 0.002 to 0.25 mmol m^{-2} d^{-1} (Abril and Iversen, 2002; Martens and Klump, 1980; Sansone et al., 1998; Zhang et al., 2008; Borges and April, 2012; Martens et al., 1998). The methane fluxes derived from our core incubations (0.1-2.6 mmol m^{-2} d^{-1}, ignoring the potentially biased value of 19.9 mmol m^{-2} d^{-1}) were high compared to these published fluxes. Our fluxes are consistent with fluxes based on porewater gradients by Thang et al. (2013) that were between 0.3 and 1.1 mmol m^{-2} d^{-1} at 3 nearby stations measured in May 2009.

A conspicuous property of all porewater profiles at station H6, with the exception of the February 2013 sampling period, was the absence of a ~~concave upward~~ curvature in the methane concentration profiles, which would be expected for net methane oxidation by aerobic and anaerobic methane oxidation (Martens et al., 1998). Most concentration profiles of sulfate and methane at Station H6 overlapped without a significant change in the methane concentration gradient. A similar observation has been made earlier for other Himmerfjärden sediments (Thang et al., 2013), and has also been reported for sediments of the northwestern Black Sea shelf (Knab et al., 2009) and in organic-rich shelf sediment of the Namibian upwelling system (Brüchert et al., 2009). Inefficient methane oxidation is also evident from the diffusive fluxes, which showed that the upward fluxes of methane into the sulfate-methane transition zone were only marginally higher than the methane fluxes to the sediment surface indicating little attenuation of the methane flux in the sulfate-methane transition zone (Table 2). One possible explanation for this phenomenon is therefore that rates of sulfate reduction–coupled anaerobic methane oxidation, except for the winter months, were low compared to the total sulfate reduction rate. An alternative explanation of our observations could be that the methane concentration gradients were affected by the presence of rising methane bubbles (Haeckel et al., 2007), or that bioturbation and bioirrigation linearized the concentration profiles (Dale et al., 2013). However, we do not favor this interpretation because of the absence of large macrofauna at station H6, the fact that methane concentrations were below the in-situ saturation concentration of methane, and the fast porewater methane sampling method.

An analysis of the cumulative distribution of ^{35}S-SRR with depth at station H6 provides clues to the proportion of organoclastic relative to anaerobic methane oxidation-coupled sulfate reduction at Station H6 (Figure 6 e-h). The gradient in organoclastic sulfate reduction is well described by ~~an~~the exponential ~~law~~function,

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$$^{35}\mathrm{SRR} = y\, z^{-b} \qquad (6)$$

where z is depth (cm) and y and b are regression coefficients (Jørgensen and Parkes, 2010). For the sediments investigated here, the exponential coefficient b varied between 0.4 and 0.9 at station B1 and 0.3 and 0.8 at Station H6 (Table 4). At Station H6 the lowest coefficient was found for February 2013, when sulfate penetrated the deepest into the sediment. Since the upward flux of methane provides an additional energy source to sulfate-reducing bacteria, sulfate reduction rates are expected to increase in the sulfate-methane transition zone. If substantial AOM-coupled and organiclastic sulfate reduction occur at the same depths the total ^{35}S-sulfate reduction rate depth gradient will be lower and the exponential coefficient b will be smaller than for a setting without AOM.~~The net effect of a substantial AOM contribution to total sulfate reduction is a low exponential coefficient b because the depth~~

~~gradient in the ^{35}S-sulfate reduction rate~~rates ~~is reduced. and higher sulfate reduction rates persist to greater depth~~. The difference between the exponential coefficients ~~of~~for the different ~~sampling periods~~observation times can be used to calculate the variation in the contribution of AOM to the total sulfate reduction rate. At station H6, between 5 % (August 2012) and 20% (April 2012) of the total sulfate reduction can be associated with anaerobic methane oxidation. A comparison of the above method with the ~~integrated~~ ^{35}S-sulfate reduction rates integrated over the length of the H6 sediment cores with the rates integrated ~~over~~in the AOM zone also indicated that >20% of sulfate ~~respiration~~reduction at H6 was ~~fuelled~~supported by anaerobic methane oxidation (Table 2). In near-shore continental margin sediments worldwide, the fraction of methane-driven sulfate reduction varies between locations and accounts for 3-40% of total ~~SRR~~sulfate reduction, with 10% possibly representing a global mean value (Jørgensen and Kasten, 2006). The average 20% contribution calculated here falls in the upper range of these values and is similar to values reported before for one of the monitoring stations within Himmerfjärden (Thang et al., 2013) and also for a very productive Chilean slope sediment (8-24 %) (Treude et al 2005b). The good match between the upward fluxes of methane in the sulfate-methane transition zone and the measured sulfate reduction rates in the transition zone also indicate that other proposed electron acceptors for anaerobic methane oxidation such as iron are unimportant in these sediments (Beal et al., 2009; Egger et al. 2014).

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4.4 Temporal variability in hydrostatic pressure

The abrupt decrease in porewater methane concentrations from November 2012 to late January/early February 2013 and the subsequent increase in April 2013 cannot be explained by variation in methane oxidation alone, because the temporal change in porewater methane concentration was large compared to the inferred methane oxidation rates based on fluxes in and out of the AOM zone. In addition, except for downward-diffusing sulfate, there was no significant other electron acceptor present at depth. It is unlikely that rates of methanogenesis would have decreased significantly between the fall and the winter and resumed again in the spring~~,~~ because of the sedimentological characteristics described above

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and the small difference in sediment temperatures ~~were similar in~~for February and April (Table 1).

Changes in organic matter sedimentation at the sediment surface also have no significant influence on methanogenesis in buried sediment and cannot explain the sudden decrease in methane concentration at depth. An alternative explanation for the changes in methane concentrations is required. A possible explanation could be that changes in upward transport of methane ~~changes~~ are due to variability in hydrostatic pressure and the associated diffusive and advective upward transport of methane from depth. The free gas depth of methane is thought to follow changes in hydrostatic pressure and temperature (Mogollon et al., 2011; Toth et al., 2015). An estimated 10% of the fine-grained sediments in the Stockholm archipelago area ~~is~~are underlain by pockets of free methane (Persson and Jonsson, 2000) and these free gas pockets are preferentially located in areas with the thickest postglacial mud accumulation, generally in the center of the sub-basins and along fault lineaments (Söderberg and Floden, 1992). Based on sub-bottom echosounder profiling, the surface of the free gas zone in accumulation areas in Himmerfjärden and other areas of the Stockholm archipelago is between 1 and 3 meter depth~~.~~ (Söderberg and Floden, 1991). During low sealevel stand the free gas zone is expected to migrate closer to the sediment surface, whereas during high sealevel the free gas zone is depressed into the sediment. The total variation in sealevel ~~may~~is related to air pressure, prevailing wind directions, precipitation, and the balance of saltwater entry through the Danish straits and freshwater discharge from rivers entering the Baltic Sea (Andersson, 2002). Additional effects are caused by local coastal bathymetry, current flow, and, possibly, and local submarine groundwater discharge. These multiple parameters result in complex subsurface hydrology and may produce sealevel fluctuations that can be as much as 50 cm, sufficient to explain the changes in methane concentrations observed here.

Unfortunately, local data within Himmerfjärden on sealevel ~~fluctuation is~~fluctuations are not available for our respective sampling locations, and ~~general~~regional sealevel stands should not be directly applied to the sample sites.

The above discussion demonstrates that a variety of processes interact in these fjord sediments to produce the observed methane fluxes. It is beyond the scope of this paper to develop a unifying model

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against which the variability of the observed fluxes can be tested, but we would like to point out that the local coastal hydrography and hydrogeology would need to be accounted for in such a coupled physical biogeochemical model. To our knowledge, sufficient subsurface geophysical data are currently not available to establish appropriate physical boundary conditions for such a model. Detailed geophysical analysis of the subsurface structure at high vertical resolution together with long-term monitoring of the porewater chemistry would shed new light on the coupling between subsurface hydrology and methane emissions.

~~The above discussion demonstrates that a variety of processes interact in these fjord sediments to produce the observed methane fluxes. It is beyond the scope of this paper to develop a model against which the observed fluxes can be tested, but we would like to point out that local coastal hydrography and hydrogeology would need to be accounted for in such a coupled physical biogeochemical model. To our knowledge sufficient subsurface geophysical data are currently not available to establish appropriate physical boundary conditions for such a model.~~

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5 Conclusions

A greater understanding of methane emissions from estuarine and coastal sediments is important to estimate the contribution of these environments to global marine methane fluxes. High benthic fluxes of methane from these sediments showed that total methane oxidation was relatively inefficient, despite the fact that anaerobic methane oxidation contributed up to 20% to total sulfate reduction. ~~High benthic fluxes of methane from these sediments showed that aerobic and anaerobic methane oxidation rates are relatively inefficient, while still contributing up to 20% to total sulfate reduction. Higher bottom water oxygen concentrations in winter played a pivotal role in methane removal in these sediments.~~ Of the different environmental regulators, bottom water oxygen had the strongest influence for the regulation of methane emissions. Oxygen availability directly enhanced aerobic organic matter mineralization by shifting the redox cascade in the sediments and indirectly by stimulating meiofauna and macrofauna activity thereby stimulating both the aerobic carbon mineralization and oxidative recycling of sulfate.

The annual variability in sediment methane concentrations and benthic methane fluxes indicate that the annual environmental changes at these near-shore, but relatively deep-water localities are considerable. Very few data on sediment biogeochemical processes are currently available for aerobic and anaerobic carbon mineralization and methane cycling during winter months when ice cover inhibits access and sampling. Process rates inferred from sampling during open-water conditions over the whole year are therefore likely overestimates.

Hydrostatic pressure changes and complex subsurface hydrological conditions may also affect the temporal variability of subsurface methane concentrations. The spatial and temporal variability of these conditions must also be considered as an important component for understanding methane emissions from near-shore coastal and estuarine waters.

~~In addition, Complex local hydrological conditions that are difficult to capture with one-dimensional transport models may affect the advective recharge of subsurface methane. These processes should also be considered as an important transport component in deeper near-shore waters.~~

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6. Author contribution

Joanna E. Sawicka conducted the sampling and analysis for the study and wrote the manuscript. Volker Brüchert devised the study, interpreted the data, created the figures and tables, and wrote the manuscript.

7. Data availability

The data are available from the second author upon request.

8. Acknowledgments

We are grateful to the staff of Askö Laboratory for their help and cooperation during the cruises and our stays on the island of Askö. We would like to thank Barbara Deutsch, Camilla Olsson and Stefano Bonaglia for their help during sampling. The study was funded by the grant from the Bolin Centre for Climate Research, Baltic Ecosystem Adaptive management (BEAM), and the EU BONUS project Baltic Gas.

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Table 1. Main site characteristics of the sampling stations.

Station	Sampling time	Water depth (m)	Temperature (°C)	Bottom water salinity (‰)	Bottom water Oxygen (µM)	Surface organic carbon (%)
B1 58°48'18’’N 17°37'52’’E	April 2012	41	2.4	6.5	160	6.0
	August 2012		6.9	7.0	260	5.2
	October 2012		6.8	7.0	224	5.1
	February 2013		3.4	7.0	380	5.0
H6 59°04'08’’N 17°40'63’’E	April 2012	39.5	1.8	5.9	40	4.6
	August 2012		6.7	6.4	150	5.1
	October 2012		9.4	6.5	191	5.2
	February 2013		1.8	5.4	300	4.7

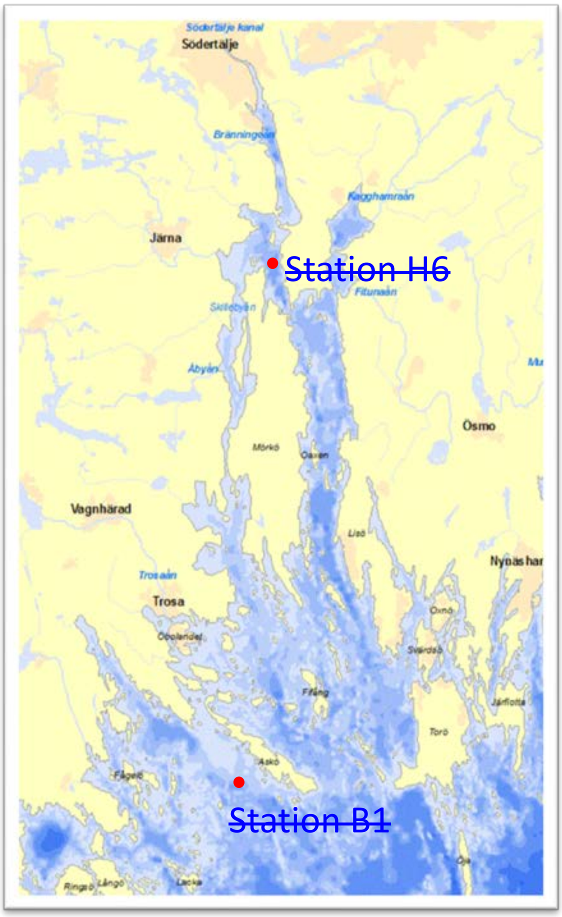
Table 2. Summary of CH_4 and SO_4^{2-} fluxes, depth-integrated ^{35}SRR, and total oxygen uptake (TOU).

Station	**Sampling time**	**Flux (mmol m^{-2} d^{-1})**						
		TOU **whole core incubation (n=4)**	**CH_4** **whole core incubation (n=4)**	**CH_4** **Diffusive flux out of sediment (n=1)**	**CH_4** **Diffusive flux into SMTZ (n=1)[2]**	**SO_4^{2-}** **Diffusive flux into sediment (n=1)**	**^{35}S-SRR integrated over AOM[3] zone (n=3)**	**Integrated ^{35}S-SRR (n=3)**
B1	April 2012	-19.7	1.2	1.6		-0.4	no AOM zone[4]	-2.3
	August 2012	-22.5	1.2	no data		-0.8	no AOM zone[4]	-0.5
	October 2012	-21.1	1.9	1.9		-1.3	no AOM zone[4]	-2.0
	January 2013	-12.0	0.1	0.1		-0.2	no AOM zone[4]	-2.2
H6	April 2012/13	-23.5	3.9[1]	2.2	2.8	-2.6	(10-15 cm)= 2.8	-11.6
	August 2012	-26.9	19.9[5]	2.4	2.6	-2.5	(10-15 cm) = 2.8	-11.7
	October 2012	-25.9	1.8	1.8	1.9	-2.6	(10-15 cm)=2.4	-11.5
	January 2013	-14.9	1.7	0.1	0.4	-1.3	no AOM zone[3]	-9.2

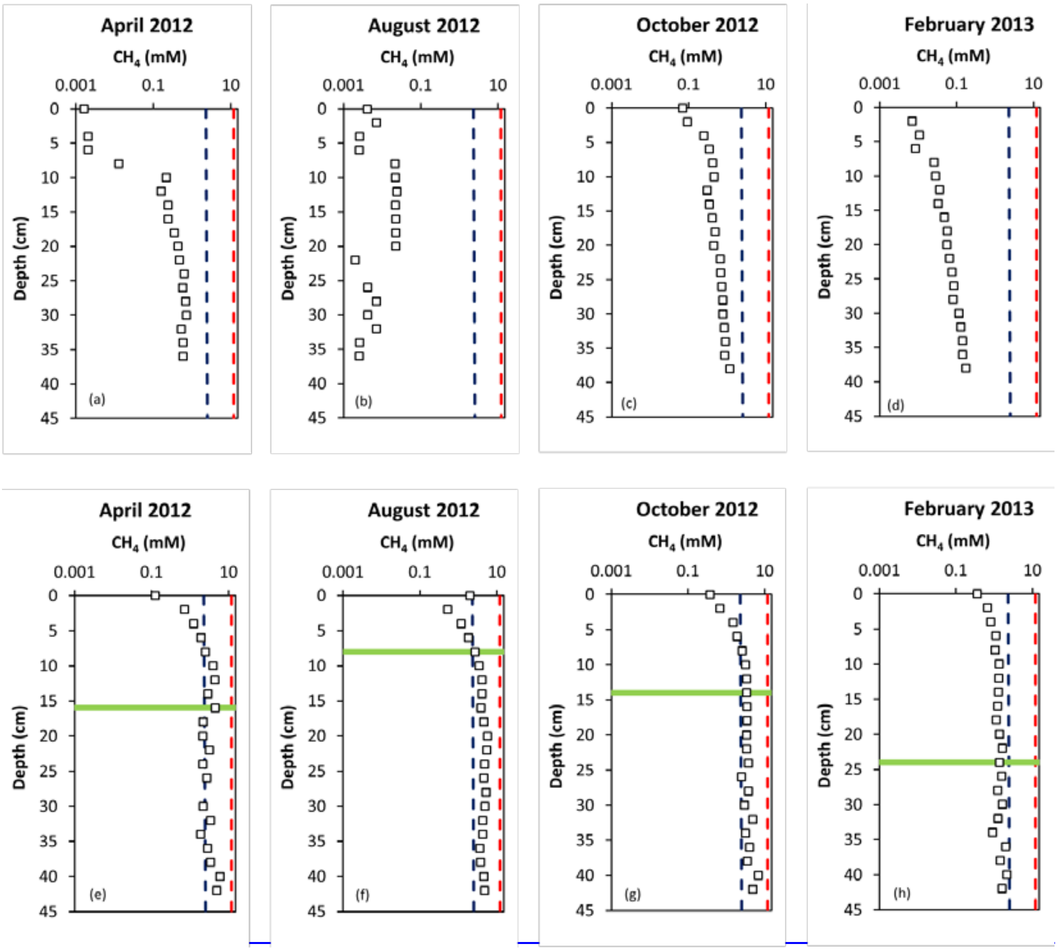
[1] whole core incubation was performed in April 2013; Diffusive fluxes were calculated for samples collected in April 2012; [2] SMTZ - sulfate methane transition zone, [3]AOM zone – zone of anaerobic oxidation of methane, [4] no AOM zone means that AOM zone was probably deeper than the core length; [5] potentially elevated due to depressurization/ex-solution effect during core incubation at atmospheric pressure;

Table 3. Best-fit regression coefficients a and b for the depth gradient of sulfate reduction rates (^{35}SRR = az^{-b} (z =depth, cm)).

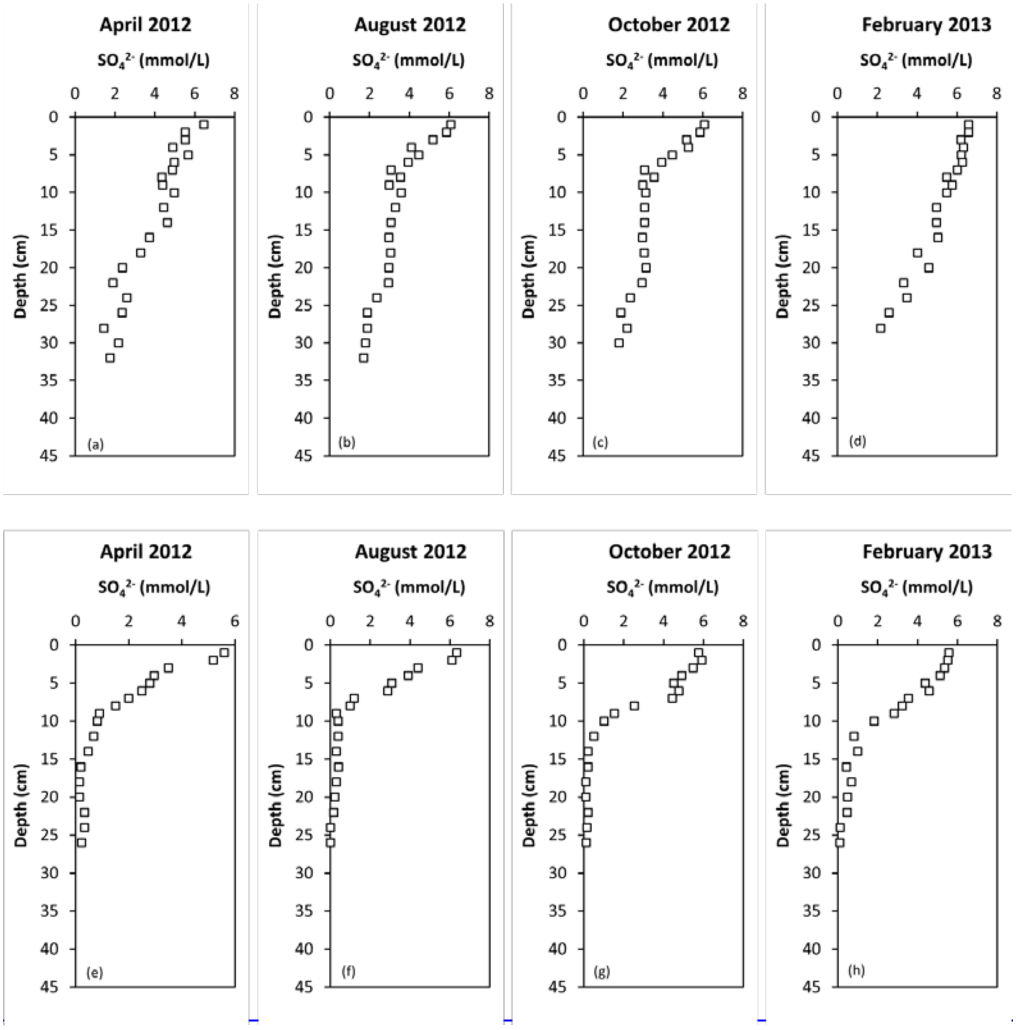
Station	**Sampling time**	**Exponential coefficient (a)**	**Exponential coefficient (b)**
B1	April 2012	147.0	-1.4
	August 2012	11.7	-0.9
	October 2012	16.0	-0.4
	February 2013	33.5	-0.8
H6	April 2012	18.6	-0.5
	August 2012	37.4	-0.5
	October 2012	133.2	-0.8
	February 2013	25.0	-0.4



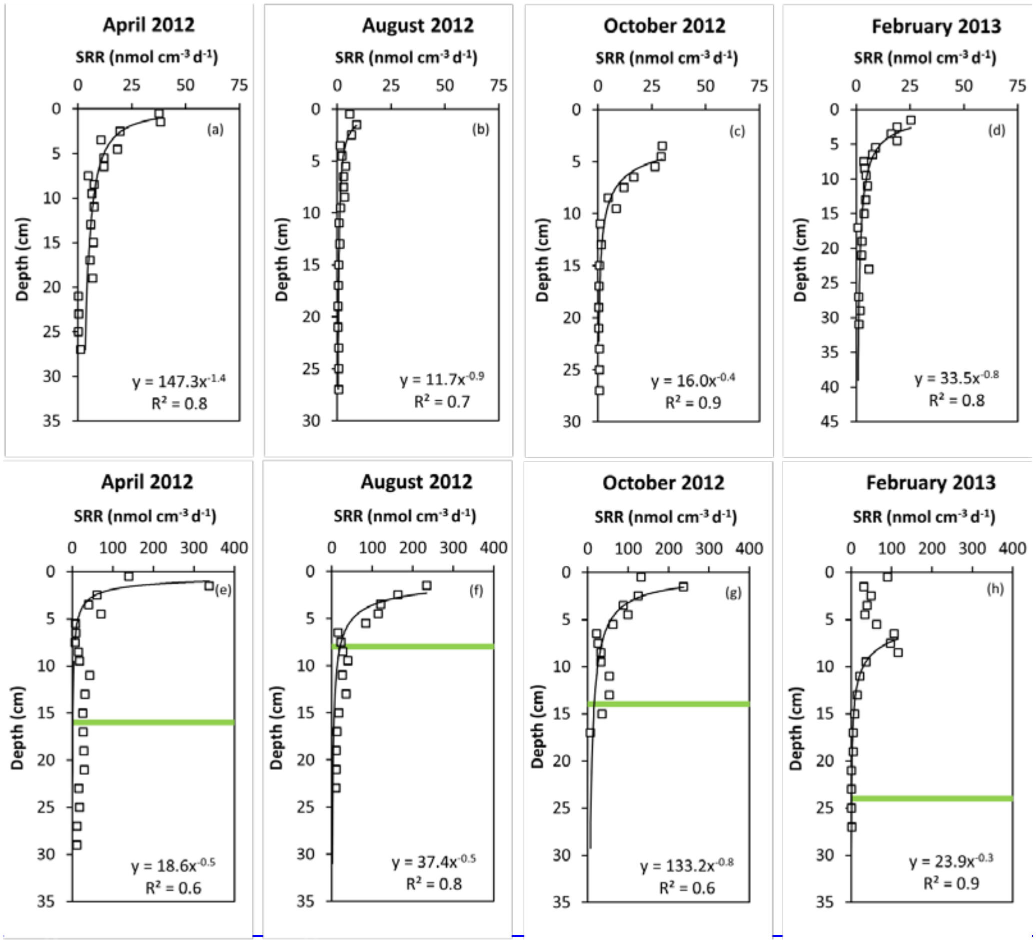
~~Figure 1. Location of sampling sites in Himmerfjärden, Stockholm Archipelago, Sweden. Detailed studies were conducted at two sites, an open water site (Station B1) and in the inner part of the estuary (Station H6).~~



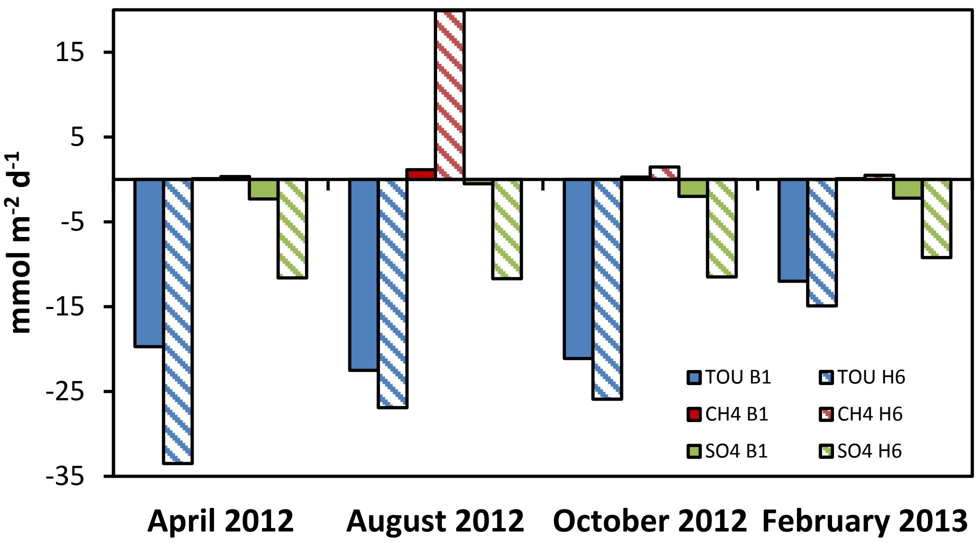
~~Figure 2. Porewater profiles of total methane at Station B1 (a-d) and Station H6 (e-h) for the different sampling periods. The green line marks the maximum depth of sulfate penetration. The dashed lines indicate the methane saturation concentration at 1 atm pressure (grey) and at the seafloor hydrostatic pressure (red) at the time of sampling.~~



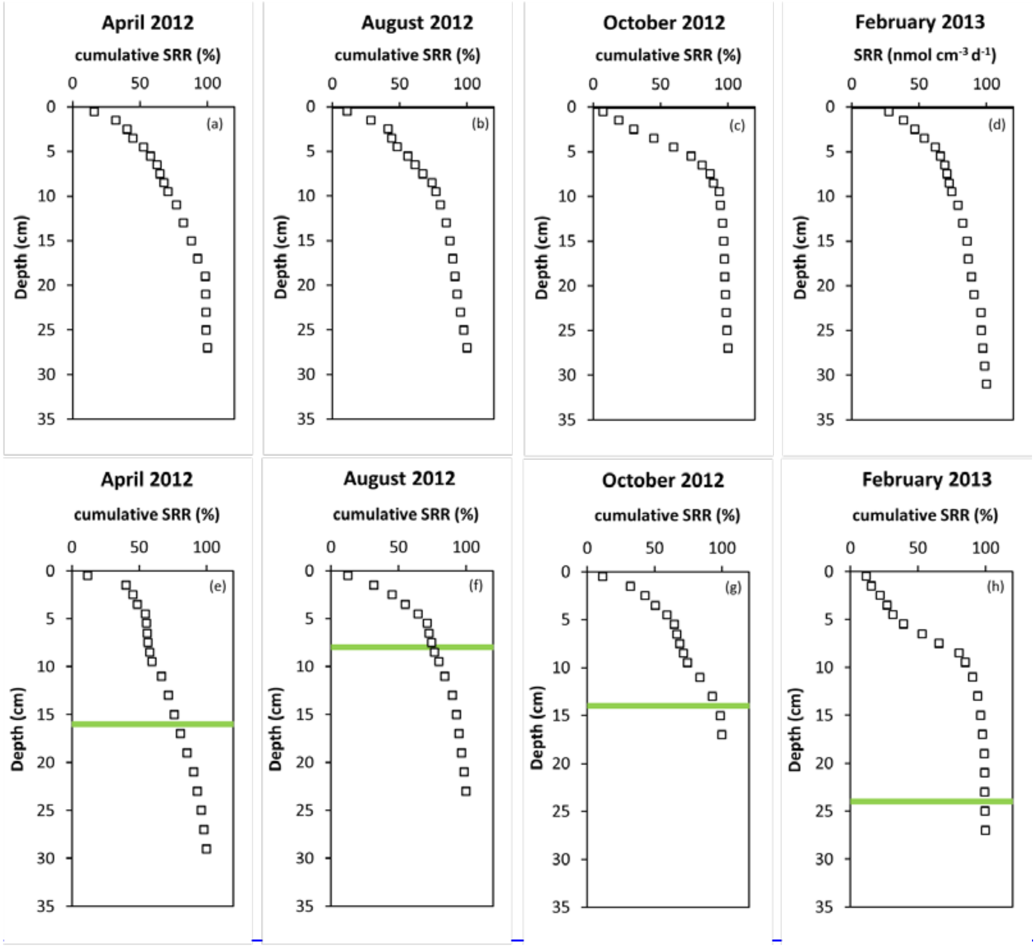
~~Figure 3. Porewater profiles of dissolved sulfate at Station B1 (a-d) and Station H6 (e-h) for the different sampling periods.~~



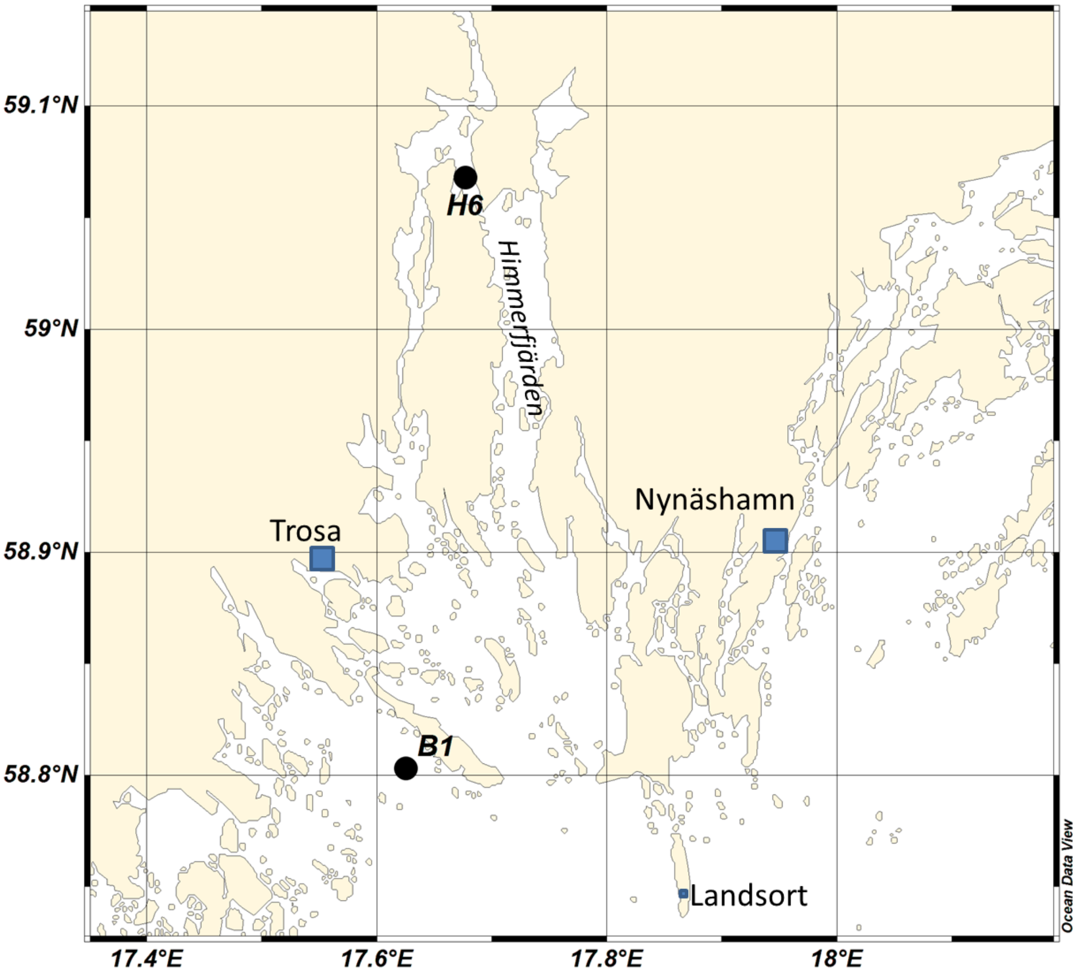
~~Figure 4. Depth gradients of bacterial sulfate reduction rates (SRR) measured with ^{35}S-sulfate. Black lines show the regression results to a power law of the form y = ax^{-b}. The green line marks the maximum depth of sulfate penetration.~~



~~Figure 5. Comparison of benthic fluxes (mmol m^{-2} d^{-1}) for sulfate (SO$_4$), methane (CH$_4$), and oxygen for the different sampling periods.~~



~~Figure 6. Depth distribution of sulfate reduction rate expressed as cumulative percentage. The green line marks the maximum depth of sulfate penetration.~~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Landsort
Ocean Data View

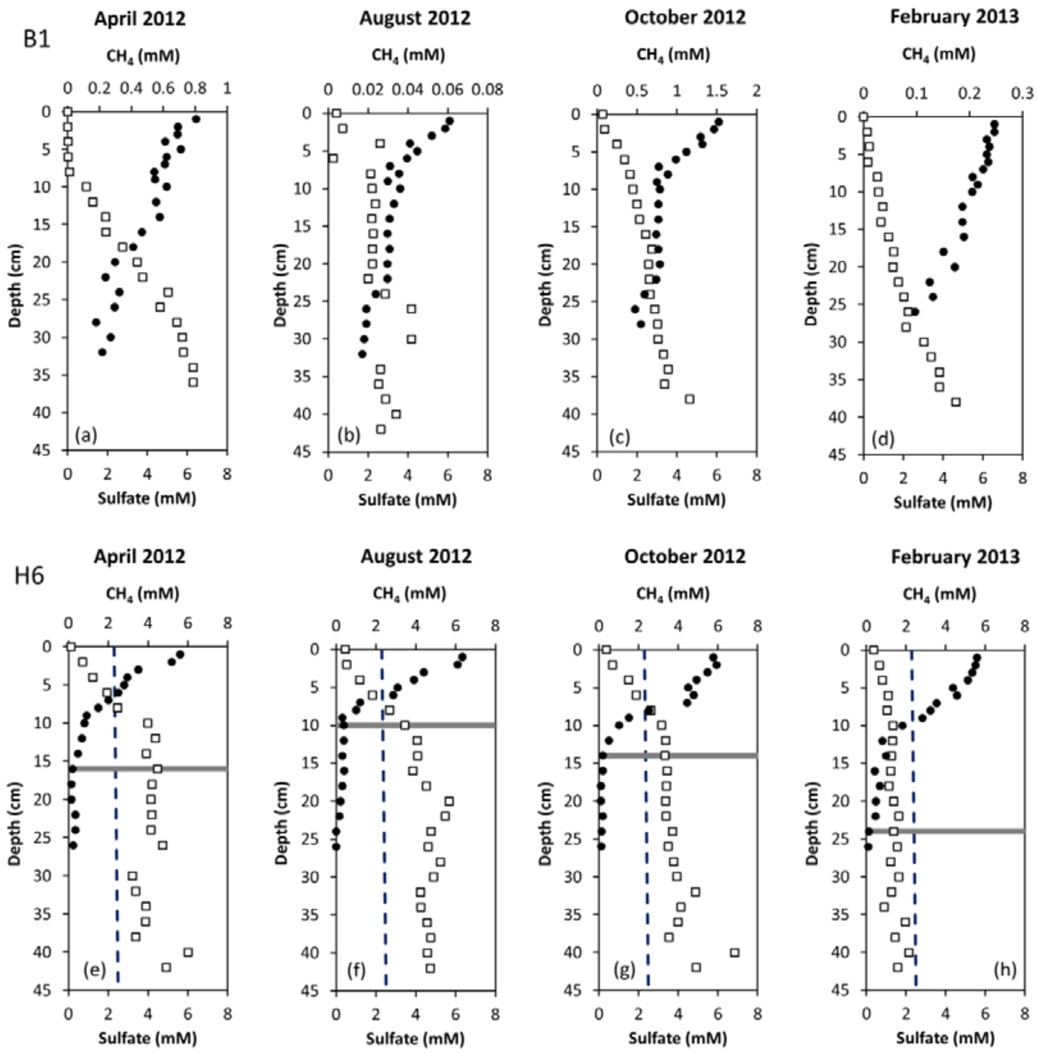

Figure 2. Porewater profiles of total methane and sulfate at Station B1 (a-d) and Station H6 (e-h) for the different sampling periods. The grey line marks the maximum depth of sulfate penetration. The dashed lines indicate the methane saturation concentration at 1 atm pressure (grey) at the time of sampling. All concentrations of methane are below the in situ saturation concentration of methane (see text for details).

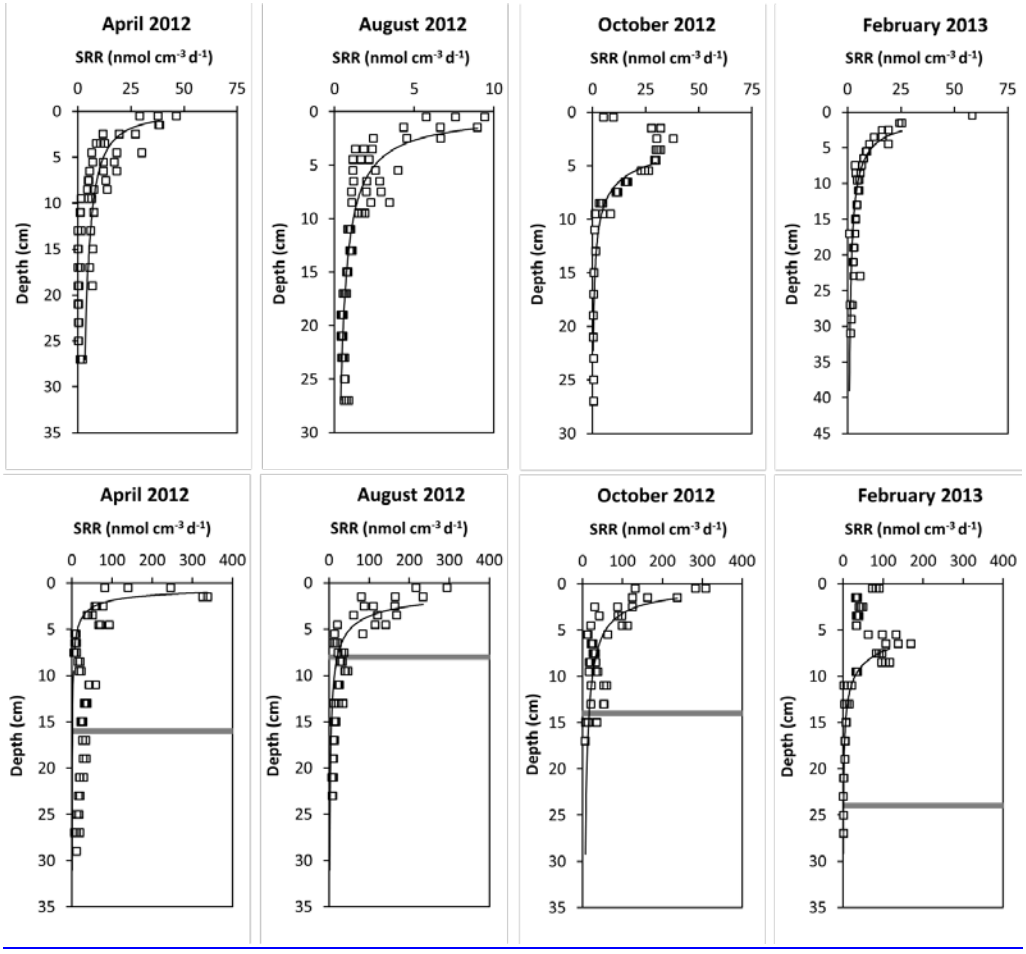

Figure 4. Depth gradients of bacterial sulfate reduction rates (SRR) measured with ^{35}S-sulfate at Station B1 (a-d) and Station H6 (e-h) for the different sampling periods. Black lines show the regression results to a power function of the form y = ax^{-b}. The grey line marks the maximum depth of sulfate penetration.

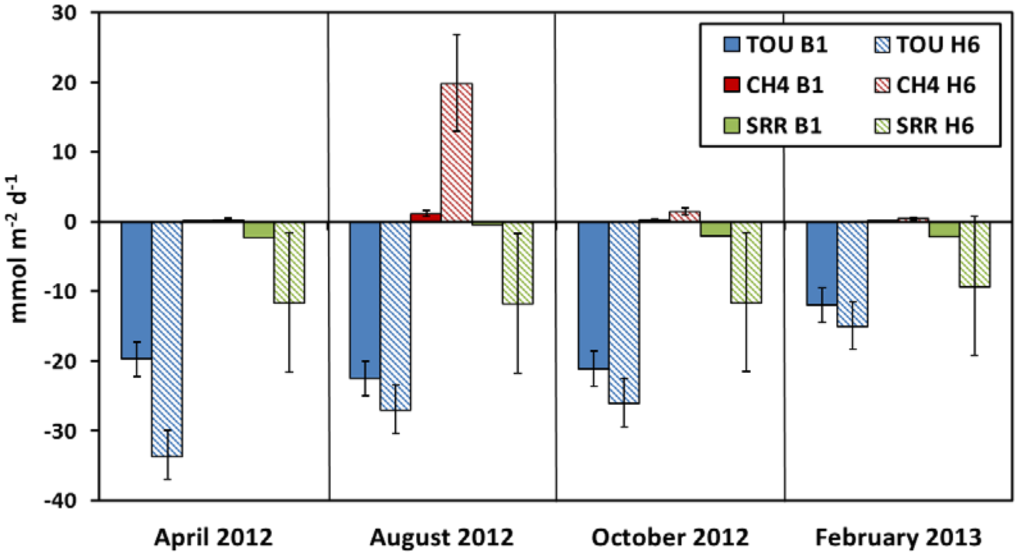

Figure 5. Comparison of benthic fluxes (mmol m^{-2} d^{-1}) for sulfate (SO_4), methane (CH_4), and oxygen (TOU) for the different sampling periods.

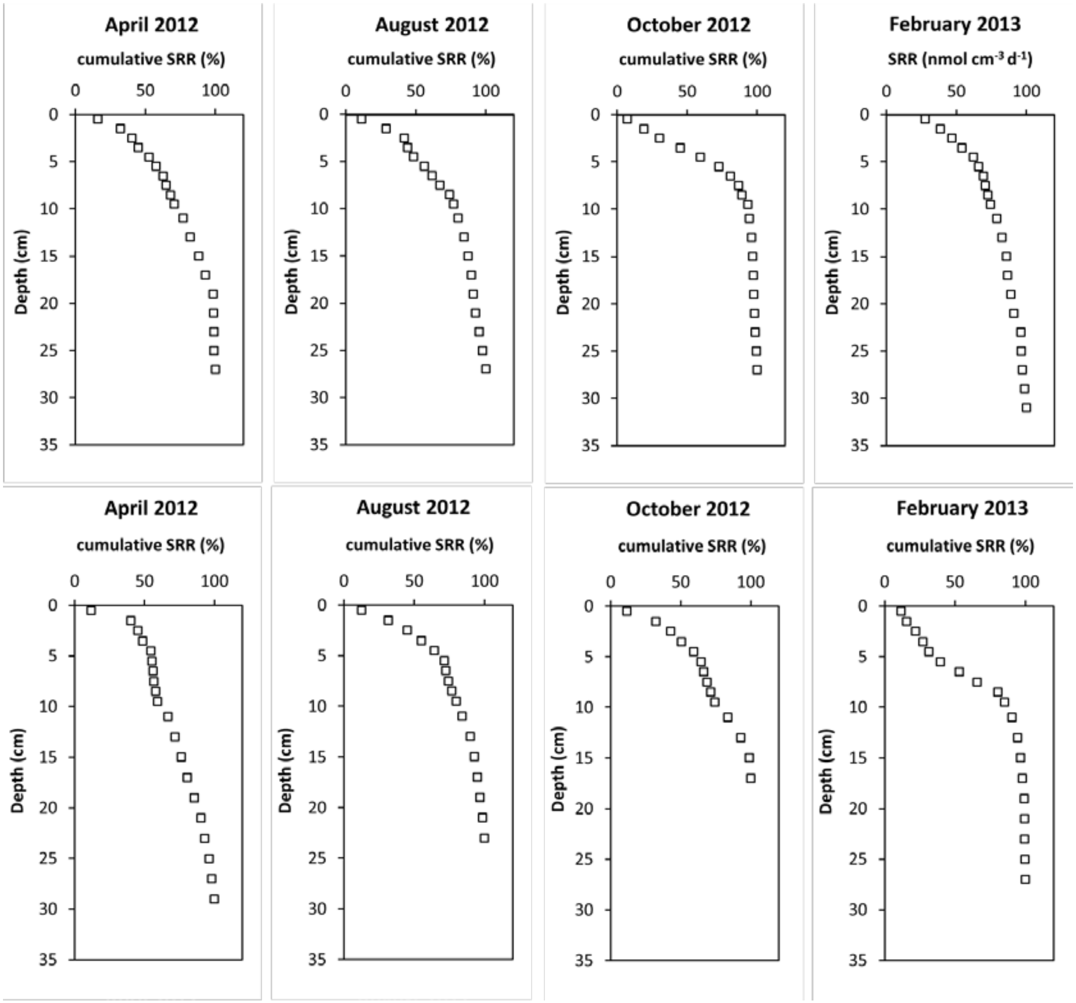

Figure 6. Depth distribution of sulfate reduction rate expressed as cumulative percentage at Station B1 (a-d) and Station H6 (e-h) for the different sampling periods.. The grey line marks the maximum depth of sulfate penetration.