2nd Response to reviewer “Annual Variability and regulation of methane in sulphate fluxes in Baltic Sea estuarine sediments.” by Joanna E. Sawicka and Volker Bruechert

To the editor:

Dear Tina Treude,

We have now addressed the comments made by the reviewer and changed our manuscript accordingly. Please find all our changes listed below with line indications, where the changes have been made. The indicated lines refer to the document with the track changes. We have followed the reviewer’s suggestions rather closely and agree with him on most points. We therefore feel that her/his thorough work has substantially improved the manuscript. Noteably, the reviewer pointed to several inconsistencies between text, tables and figures that forced us to return to our original raw data. It turned out that some recalculations were necessary and these are now reflected in some of the new fluxes given in Table 2. It turned out that the discrepancy between the whole-core methane fluxes and the fluxes based on concentration gradients differed at both stations, which we acknowledge in the text. It is unfortunate that the whole-core fluxes do not agree better with the diffusive fluxes at the low-methane station, and this, as we state in the text, had possibly to do with some tough sampling conditions on a small vessel. However, we still feel that our discussion, which is mainly grounded in the porewater and rate data, is not affected by the methane whole-core flux data, and our overall conclusion holds. We would like to explicitly address the fact that we do not provide a fully integrated model of our data. We feel that even without the model our data are a significant scientific contribution. This study remains one of the few from this high latitude and in particular from the low-salinity regions of the Baltic Sea. It provides substantial amount of new data for near-shore coastal sediment.

We hope that our revised version sufficiently satisfies the expectations of you and reviewer.

Sincerely and best regards,

Volker Brüchert and Joanna Sawicka

Lines refer to the version with track changes.

Line 60: remove “potentially”
A: removed

Line 66: In the water methane cannot be produced in the sediment, please rephrase.

A: The sentence now reads as follows: line 68
“In estuarine waters methane can be derived from underlying anoxic sediments, transported laterally due to freshwater or sewage discharge, seepage of methane-rich groundwater, or it can be derived from near-shore aquatic plants (Borges and Abril, 2011).”

Line 113 and 423: you state that 23% of the freshwater is from a river, but also that there is no important river entering the system.

A: 23% of total freshwater discharge came from the river Trosaån. However, this is still a very minor volume compared to the water exchange with the open Baltic. The average annual freshwater discharge given in Larsson (2013) is about 23 $m^3 s^{-1}$ from all freshwater sources and the total volume of the estuary given in Engqvist and Omstedt (1992) is 2968 x 10^6 m^3. This translates into a turnover time give the freshwater discharge-based exchange time of 4 years. This is considerably slower than the exchange with the open Baltic (140 days bottom water and 70 days surface water) cited in Savage and Elmgren (2010) and indicated by the modest surface salinity gradient across the N-S section of Himmerfjärden.

Revised text: from line 113:
It is morphologically characterized by four basins, divided by sills and has a low flushing rate (~0.025/day) (Savage and Elmgren, 2010). The freshwater discharge is small compared to the exchange with the open Baltic and was estimated to be 23 m^3/s on average in 2012 comprising land run-off and precipitation (30% and 21% respectively), outflow from Lake Mälaren in the north (19%) and the river Trosaån (23%), and discharge from a sewage treatment plant (6%) (Larsson et al., 2012). The sewage treatment plant, built in the early 1970s, treats sewage water from 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).

Line 134: where are the April 2013 data?

A: They are shown in Table 2.

Line 155: what depth are the corg data from?

A: Inserted ” for the topmost cm of sediment”

Line 156/7 delete one mention of “freeze dried sediment”
A: Removed

Line 159: It still lists 2 hours drying time even though the author answered that the actual drying time was longer;
A: We do not have an exact record for how long the drying period exactly was, but the sediment was certainly dry. Time period has been removed from the text.

Line 168: delete “exactly”
A: Done

Line 170: the authors still do not cite a reference for fixation of the samples with 5M NaCl, even though they say it is a widely used method;

A: Added reference: Thang et al. (2013)

Line 170: now it sounds you measure the sample immediately after injection of the brine.

A: The text reads as follows:
A sediment sample of 2.5 mL was taken with a 3 mL 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 (Thang et al., 2013). Before analysis, the sample was shaken and 5 mL of brine was injected into a sample vial to displace 5 mL gas 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) after separation on a 3 feet Porapak Q pre-column before a 9 feet Hayesep D column with N_2 as carrier gas.

Line 171: The authors agree that an hour is very likely does not get all the adsorbed methane. In the next answer, however, they state that the hour is actually sufficient and instead of clarifying that, the authors delete the information altogether and only state it in the response letter;

A: The reviewer misunderstood our comment. We do not think there is significant sorption after one hour. In any case, most of the analysies were performed later than one hour after sampling. The one hour period has been removed from the text.

Line 218: switch the order
Done (line 224)

Line 242/3: move "without headspace", as now it sounds that the "50ul of 50% ZnCl2" did not have a a headspace.
Done (line 248)

Line 255: which salinity did you choose the ß for?
Added: "using the salinity of the bottom water at the respective time point (Table 1)"

Line 267: answering my comment, the authors state that they did some replication on methane profiles, but do not say anything about the fact or the results in the manuscript;

A: See line 186: The reproducibility of the method has been tested at a station in the archipelago that is not part of this study by replicating methane sampling on multiple sediment cores. Concentrations were found to deviate by about 15%, likely due to shipment movement and sediment heterogeneity.

Unfortunately, the manuscript still in not organized all the way through with the same order for the stations in the results (e.g. line 300---304). Also, sometimes it seems that the authors are discussing a different data set (line 307).
A: See changes in the text: lines
A: We certainly don't discuss another data set, but we apologize for the confusion. In any case, the correct SMT depths had been shown by the grey bars.

Line 283: "sediment organic carbon"
A: Changed

Line 300ff: what is the trend in B1 and how is February B1 an exception to the H6 trend?
The trends are now described in more detail in the text.

Line 307: the graph shows the lowest sulphate penetration and steepest gradient in August;
A: They are very similar in August and October at Station B1, but we agree that at Station H6 they are slightly steeper in August than in October. See changes in text line 310-327.

Line 314: what is the trend in April and February?
A: Changed to: "Generally, the sulfate gradient was similar throughout the measured profile in April and February, whereas in August and October, sulfate decreased more steeply from the surface down to 10 cm depth."

Line 318: Graph shows 16, 14, and 10 cm (8cm in Figure 3);
A: See changes lines 310-327. The correct sulfate penetration depths are now given for Station H6.

line 319: a 100-500um oxygen gradient from bottom water to 0 gives a high flux, which could be coupled to methane oxidation. Additionally, bioturbation can provide oxygen even below this depth.

A: Yes we agree, but we are a bit confused whether this comment corresponds to the line the reviewer refers to.

Line 322-34: state that August is much lower than April and October

A: See changes in line 340
Inserted the following sentence: "Contrary to expectations, the lowest sulfate reduction rates were measured in August."

Line 335: Keep the order the same as in the previous paragraph.
A: The section now reads: line 350
"At Station H6, depth-integrated sulfate reduction rates varied 9.2 to 11.7 mmol m^{-2} d^{-1}. 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. In April, August, and 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."

Line 338: Please state how you define the sulphate penetration depth as otherwise your rates below it do not make any sense.
We changed the term from sulphate penetration depth to 'minimum sulphate concentration depth' and sentence in line 329f of the previous section: "The initial depth at which sulfate reached the lowest concentration from the surface down was defined as the initial minimum sulfate concentration depth, which occurred at 16 cm depth in April, 10 cm in August, 14 cm in October and at 25 cm depth in February.".

Line 338: use the abbreviations you introduced e.g. SRR
A: We changed to SRR to be consistent.

line 338: there is no sulphate in August below 10 or 8 cm, depending on the graph, so there cannot be a peak in sulphate reduction, only in potential sulphate reduction)

A: This section of the text is a description of the data after addition of 1 mM sulfate with the tracer. As we commented on previously, the idea of adding 1 mM of sulfate was to avoid bias due to potential sulfate reduction. No addition of ‘cold ’ sulfate produces difficulties in comparing sulfate reduction rates across steep sulfate gradients. This problem has been addressed in previous publications (e.g., Holmkvist et al., 2014 GCA). We disagree with the reviewer to say there is ’no’ sulfate below the SMT, because the ion chromatograph requires dilution of the sulfate sample (50 x dilution) (detection limit of the IC system 5μM), which raises the detection limit to a concentration of around 100 μM when calculating in the dilution. At this concentration there can be potentially sulfate reduction. We interpret the observed sulfate reduction increase in the SMT as a real peak in activity.

The rates in table 2 are sometime negative and some positive. At the same time the direction they are described in change. A negative TOU means that there is oxygen diffusing out of the sediment. A positive CH4 flux out of the sediment that there is methane diffusing out of the sediment, a negative SO4 into the sediment means that there is sulphate diffusing out of the sediment. Please correct as mentioned before.

A: We changed the TOU and sulfate reduction to positive values and the methane flux to negative values, and hope we have understood the reviewer correctly. We made sure there is a consistent use in text, table 2 and figure 4.

Line 352: no change in sulphate concentrations argues for nor organoclastic just as much as for no AOM sulphate reduction.

A: But the key here is that the methane concentrations do not change in gradient across this interval as would be expected if there was a significant coupling between the two due to AOM.

Line 354: yes, there is the possibility of a cryptic sulphur cycle. But there is not a good reason to expect this cycle not to go on above. Thus, if you keep this information in there, you need to discuss it in more detail and add the information to the legend of the graph;

A: We use the term potential sulfate reduction, because our data do not permit us to say otherwise. However, we would not like to expand beyond what we write here, just as much with a further discussion on potential cryptic sulfur cycling in shallower sediment layers. Short-term ^{35}S incubations alleviate part of the cryptic sulfur cycling problem compared to gradient-based methods. We would also like to emphasize that a further discussion on cryptic sulfur cycling will not change our interpretation, which is primarily concerned with the flux of methane and the efficiency of AOM.

line 367: please discuss the effect of the profile resolution on the modelling results…)

A: In response to this we added lines 285-288 in section 2.10: “Since the resolution of the porewater methane analysis was 2 cm, steep concentration changes below this resolution cannot be captured. This could lead to an overestimation of the flux across the sediment surface, e.g., due to aerobic methane oxidation in the topmost mm of sediment. Similar effects may occur in the sulfate-methane transition zone.”

Line 367: indicate which direction the methane flux is going.

A: Changed to “Upward diffusive fluxes …”

Line 374: It is hard to discuss seasonal variation with 4 data points from one year.
A: We agree, but there are distinct differences for the different time periods that fit with an interpretation of seasonal variability.

Line 383‐391: Please link the statements directly to your data.
A: line 386: Inserted: All R values calculated for pairs of temperature versus rate/flux were less than 0.2 …" and inserted "Table 1" at the end of line 391.

Line 397: there can also be imitations that occur at different temperatures, might be indirect though increased rates and thus increased competition, that can influence it. It does not only have to be a direct temperature effect.
A: Acknowledged. Since our data do not allow us to specify whether there are direct or indirect effects at the organismal level, we restrict ourselves to the term methanogenesis and methane oxidation
We changed the sentence, line 426-429: "This requires significantly higher temperature stimulation of methanogenesis than methane oxidation, the lack of an electron acceptor, or competition for the same electron acceptor used by other organisms than methane-oxidizing bacteria."

Line 402‐407: the argument is not convincing. For such small temperature differences, you do not expect to see changes in the membrane composition related to changes in temperature adaptation.

A. We agree with the reviewer, but thisis exactly the point we are making in the text.

And also closely related microbes can be psychrophiles and mesophiles and thus have a very different temperature adaptation. Your data indicate that there is not such a tight coupling between sulphate reduction and methanogenesis.
A: We think that the reviewer misinterpreted our argument. Our argument refers to the, as we suggest, unlikely hypothesis that significant temperature adaptation differences should be expected between Archaea involved in methanogenesis and Archaea involved in anaerobic methane oxidation. However, as the reviewer wrote above, it is possible that the AOM process is also not directly regulated by temperature adaptation of Archaea, and instead indirectly by a temperature response of syntrophic SRB.
We have therefore inserted the following sentence, line 436-438: "However, temperature control may not manifest itself by direct kinetic or bioenergetic regulation, but indirectly by influencing competing processes, e.g., sulfate reduction versus methanogenesis. "

Line 426: the percentage likely changes with season with resuspension being highest in fall, fresh organic matter being highest after a bloom and such. Thus arguing with general numbers for the whole year does not do justice to a potential effect of seasonality on the organic matter input. Also, your indicated bioturbation depth can supply fresh organic matter to deeper sediment depth, thus influencing rates not only on the surface.
A: The point that we are making is that the annual sedimentation only for a very short period of time is dominated by primary organic carbon. We also agree with the notion that bioturbation transports fresh organic matter to depth.
We modified the last sentence eof the paragraph as follows, line 468-474:

“Observations over a 5-year period by Blomqvist and Larsson (1994) indicated that primary organic carbon dominates organic sedimentation in the spring and summer at station B1, whereas station H6 is characterized by a spring term dominance of primary carbon deposition, but a much greater contribution of resuspended organic material to organic sedimentation (Blomqvist and Larsson, 1994).”

Line 460 – 461: Please provide reference or calculation.
A: The method is based on the assumption that organic matter reactivity can be described by a power law and follows a description initially proposed by Jorgensen (1979) and further used in Jorgensen and Parkes (2010) (cited). However, to our knowledge, the specific useage of this relationship for AOM superimposed on top of organoclastic sulfate reduction, is novel.

Line 485 – 488: that is not really what you see. The SRR below 10cm is lower than in other times, and thus, the effect on methanogenesis should be lower.
A: This would be true, if we ignored the methane concentration data and the sulfate penetration depths. However, our argument builds on the combined information.

Line 486: you say that iron and manganese reducers in your sediment outcompete sulphate reducers (in your response, but again not in the manuscript, where you also do not mention that usually they do not). You cite Downs and Bruchert Goldschmidt 2013 and Bonaglia etal. 2014 Biogeochemistry (which both do not say anything about sulphate reduction). What do you base your statement on?
A: In our response Bonaglia et al (2014) was used as reference for oxygen pentration depth, denitrification, anammox, and nitrate reduction with ammonium; Downs and Bruchert (2013) was used as reference for iron and manganese reduction. We don’t have the combined information in one published reference, but in different papers. Since these studies were conducted at the same time, they can be used as reference.

We removed the following sentence from the text (line 527) : “Since the concentrations of other electron acceptors (e.g. for denitrification, iron, and manganese reduction) are highest in the topmost 3 cm (Bonaglia et al., 2014; Downs and Bruchert, 2013), the depth of sulfate penetration and organic matter degradation via sulfate shifts deeper in the sediment, which confines methane production to deeper layers.”

Comment to previous comment line 465: Line 503 does not state what you mention in the answer, and I cannot find it. Additionally, it would be much better to get some numbers instead of just “fit well”.

We are sorry, but our response referred to line 563, not line 503:

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?

Pleas see text (lines 589-594)
“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 35S-sulfate reduction rates integrated over the H6 sediment cores with the rates integrated over the AOM zone also indicated that >20% of sulfate respiration at H6 was fuelled by methane (Table 2).

Comment to previous comment line 497---503: The authors agree that if it is hydrostatic than it is not seasonal. Additionally, they only have the data for one year, so it is hard to say which of the changes are clearly seasonal and which just variations over time. But they still argue for a seasonal signal.
A. We agree that more data are needed to explore the effects of hydrostatic pressure changes, but this is what we write in the text.

Comment to previous comment line 519---520: You data do not "indicate that hydrostatic changes or changes in pore water advection may have a considerable influence". Your data indicates that the parameters you measured do not alone control the variations and you discuss, without presenting data or modelling, that it is hydrostatic changes and pore water advection.

A: We do not agree that we should not discuss the possibility that hydrostatic pressure changes and advection affect the methane concentration and fluxes. Our data should not be misunderstood that we favor one or the other hypothesis. But to leave our data without providing a possible scenario that could help to explain the variability does not seem correct. We are sorry but we cannot provide a encompassing model for the reasons presented in the previous discussion round. See our discussion lines 628-633 and 637-645.

Copy from our first response letter:
A: 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.

Table 2: how did you determine the AOM zone?
A: The AOM zone was defined a) from the overlap between sulfate and methane; b) an increase in sulfate reduction rates above the rates measured in the depth intervals above;

Map: the map still is not very helpful if plotted in black and white
A: We have changed the map one more time and also provide an insert of the greater Baltic Sea region.

Figures 2. 3. 5: Please delete the repeat mentioning of the months in the middle.
A: We removed the months.

Figure 2: please provide legend showing which is CH4 and which is SO4. Change CH4 scale in (h) so the trend is more clear.

A:We added a legend for sulfate and methane in in panel (h). We would like to retain the same x-scale as in the other panels for H6

Figure 3: (a) and (b) seem to show a second peak around 8cm.
Statistically this is difficult to support. Two of the injections showed higher rates, the third one didn't.

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

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Field Code Changed

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 versus 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 m^{-2}d^{-1} in winter at an open coastal site to 2.6 mmol 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. The 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 were 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.

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

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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 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 produc~~can be derive~~e~~d ~~by methanogenesis in~~from underlying anoxic sediments, transported laterally due to freshwater or sewage discharge, seepage of methane-rich groundwater, or it can be derived ~~transport in the near-shore by~~from near-shore 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, a volume of 2968 x 10^6 m^3, and a N-S bottom water 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 of about 0.025/day ~~and derives from~~(Savage and Elmgren, 2010). ~~In 2012,~~ The ~~the~~ freshwater discharge is small compared to the exchange with the open Baltic and was estimated to be 23 m^3/s on average in 2012 compris~~ed~~ing land run-off and precipitation (~~33~~30% and ~~14~~21%, respectively), outflow from Lake Mälaren from 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

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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).

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 that was present year round, 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.

2.3 Organic carbon concentrations and porosity

~~Surface sediment c~~Concentrations of organic carbon were determined for the topmost cm of sediment 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 more than fourtwo 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 was 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 (Thang et al., 201~~2~~3). For analysis, ~~T~~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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Formatted: Font color: Custom Color(RGB(19,20,19)) where ~~V_{head}~~$CH4_{hsp}$ 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. The reproducibility of the method has been tested at a station in the archipelago that is not part of this study by replicating methane sampling on multiple sediment cores. Concentrations in multiple cores deviated by about 15%.

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 stopcocks 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.

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. $^{35}SO_4^{2-}$

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tracer solution was diluted in a 6 ‰ NaCl solution containing 0.5 mM SO_4^{2-}. 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 to 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 chromium 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):

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

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

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 enough 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} day^{-1}.

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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 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 5 mm diameter 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 without headspace 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 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.

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~~Partial~~The partial pressure of CH_4 in the equilibrated headspace and water was calculated using the solubility coefficient β for CH_4 using the salinity of the bottom water at the respective sample time (Table 1) (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 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 calculated for the bottom water 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). Since the resolution of the porewater methane analysis was 2 cm, concentration changes below this resolution cannot be resolved. This could lead to an overestimation of the flux across the sediment surface, e.g., due to aerobic methane oxidation in the topmost mm of sediment. Similar effects may occur in the sulfate-methane transition zone.

3 Results

3.1 Bottom water temperature, dissolved oxygen, sediment 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

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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.

3.2 Methane and sulfate concentrations

At both stations, 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. At station B1, the highest methane concentrations in the sediment cores were recorded in October 2012, when they reached 0.9 mM (Figure 2a-d). Surprisingly, the lowest methane concentrations were recorded in August 2012. This was possibly due to drift of the vessel during sampling in rough seas at that time into an area underlain by neighbouring glacial clays with low porewater methane concentrations. Excluding the August data, methane concentrations were low and between 1 and 10 µM ~~to~~to a depth of 6 cm, 2 cm, and 6 cm ~~depth~~ in April, October, and February, respectively, before they increased sharply~~ with depth~~. At station H6, the highest and lowest concentrations in the cored depth interval were 5.7 mM and ~~1.5 mM at station H6 and~~ 1.5 mM, and recorded in August and February 2013, respectively. At this station, the methane concentrations generally increased linearly from the surface down to 10 cm depth. Below this depth they only increased slightly or remained constant.

Sulfate concentration gradients changed between the different seasons at both stations reflecting changes in sulfate reduction rates over the observation period. At both stations, the sulfate concentration gradients were steepest in the topmost 8-10 cm ~~were steepest~~ in August~~October~~, intermediate in April~~ and August~~ and October, and lowest in February indicating highest and lowest sulfate reduction rates in ~~October~~late summer and ~~February~~winter, 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~~

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~~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. In August and October, a distinct decrease in the sulfate concentration gradient occurred at around 8-10 cm depth. Despite some variability in the sulfate concentration profiles, the sulfate concentrations at the bottom of the core were similar during all observation periods. At station H6, sulfate always reached minimum concentrations of less than 100 µM in the cored sediment interval, albeit at substantially greater depth in February. ~~Depletion~~The initial depth at which sulfate reached the lowest concentration from the surface down was defined as the initial minimum sulfate concentration depth, which ~~already~~ occurred at ~~5~~16 cm depth in April, 10 cm in August, ~~and~~14 cm in October and at ~~9~~25 cm depth in ~~August~~February ~~sulfate concentrations showed a typical concave gradient~~.

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, the depth-integrated ~~sulfate reduction rates~~SRR over the cored depth varied from 0.5 to 2.3 mmol m^{2} d^{-1}. The depth-resolved 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). Contrary to expectations, the lowest SRR were measured in August, which was possibly also due to the fact that the vessel drifted into a glacial clay area. 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 depth of maximum SRR, rates decreased exponentially indicating that organoclastic sulfate reduction dominated and that the reactivity of the degrading organic material decreased exponentially with depth. More than 90% of the integrated sulfate reduction took place in the top 15 cm of sediment (Figure 5 a-d). Over the cored sediment interval, there was no peak that could be attributed to significant AOM. Nevertheless, the distinct curvature of the methane concentration profile

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in February 2013 at station B1 suggests that methane was oxidized in the sulfate reduction zone and that some of the sulfate reduction may have been coupled to anaerobic methane oxidation. At Station H6, depth-integrated SRR varied 9.2 to 11.7 mmol m^{-2} d^{-1}. ~~t~~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~~In April, August, and October 2012 two distinct sulfate reduction rate peaks were found at station H6, one at the surface, and a second peak between ~~10~~10 cm and 1~~5~~8 cm depth. The latter peak covers 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. We therefore defined the depth interval near the minimum sulfate concentration depth together with elevated SRR as the AOM zone (Table 2). ~~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 (Thang et al., 2013; Wegener et al., 2012). The depth-integrated rates of ~~sulfate reduction~~ ~~^{35}~~SRR in the 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 elevated rates ~~coupled to methane oxidation~~between 10 and 18 cm depth were not visible~~.~~, although another 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. Some ~~S~~sulfate reduction was also detected below 18 cm depth at station H6 in April, August, and October. Since non-radioactive carrier sulfate was added to the ^{35}S-tracer during

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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).~~

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 4. Total oxygen uptake was lowest in February at both stations (B1: -12.0~~±~~ ± 1.5 mmol m^{-2} d^{-1} and H6: -14.9~~±~~ ± 1.6 mmol m^{-2} d^{-1}~~)~~), and highest in August at station B1 (-22.5 ± 2.9 mmol m^{-2} d^{-1}) and in April at station H6 (-33.5~~±~~ ± 3.5 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 February to -2.7 mmol m^{-2} d^{-1} in August at station H6 (Table 2). These rates are significantly lower than the depth-integrated 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.~~09~~1±0.05 mmol m^{-2} d^{-1} (February) to -1.~~16~~2±0.6~~2~~ mmol m^{-2} d^{-1} (August) at station B1 and from -0.3±0.~~07~~1 mmol m^{-2} d^{-1} (April 2012) to -19.9±7.~~77~~8 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.~~ However, the following year, a significantly higher methane flux of 3.9 mmol m^{-2} d^{-1} was measured in April 2013 at station H6. Significant upward

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~~Diffusive~~ diffusive methane fluxes ranged from 0.02 mmol m^{-2} d^{-1} (February 2012) to ~~1.6~~0.3 mmol m^{-2} d^{-1} (~~April~~August) at Station B1 and from 0.~~4~~5 (February) to 2.~~3~~4 mmol m^{-2} d^{-1} (August) at station H6. Thus, there was a generally poor agreement between whole-core and diffusive flux-derived methane fluxes. The large discrepancy between the August 2012 diffusive flux and whole~~-~~core flux is best explained that the cores were taken from sediments with different organic carbon contents. Since several Multicorer casts were taken per station and the vessel's positioning ability in strong winds was at best tens of meters, sediment heterogeneity can possibly explain this difference. The very high whole-core flux value measured in August 2012 at Station H6 is likely due to ebullition during the incubation at ambient air pressure and oversaturation of the porewater with respect to atmospheric pressure.

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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. All R values calculated for pairs of~~The~~ temperature versus rate/flux ~~relationships~~ were ~~generally non-linear~~less than 0.2 and not consistent for the fluxes of oxygen, methane, and sulfate indicating that additional environmental 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 (Table 1). 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

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sediment is less temperature-sensitive than methanogenesis preventing methane oxidizing bacteria from keeping up with the enhanced methane flux during summer. This requires significantly higher temperature stimulation of methanogen~~s~~esis than methane oxid~~izers~~ation, the lack of an electron acceptor, or competition for the same electron acceptor used by other organisms than methane-oxidizing bacteria. 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~~directly applicable to 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). However, temperature control may not manifest itself by direct kinetic or bioenergetic regulation, but indirectly through the influence on competing processes, e.g., sulfate reduction and methanogenesis. ~~This is further discussed in section 4.3.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

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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 (Blomqvist and Larsson, 1994). The major export periods occur during the spring phytoplankton bloom after ice breakup from March-April until early May, during a late-summer cyanobacterial bloom in August, and after a weaker, 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). Observations over a 5-year period by Blomqvist and Larsson (1994) indicated that primary o~~However, only 10% to 60% of the total vertical mass flux may be composed of primary o~~rganic carbon dominates organic sedimentation in the spring and summer at station B1, whereas station H6 is characterized by a spring term dominance of primary carbon deposition, but a much greater contribution of resuspended organic material to organic sedimentation~~, while the remainder has been interpreted as resuspended material~~ during the fall (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~~y^{-1} (Thang et al., 2013; Karlsson et al., 201~~3~~0). The combined effect of these 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 2-3 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

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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 may 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~~a year. These conceptual modelling results can be tested with our Himmerfjärden data.

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)~~confining methane production to greater depths in the sediment.~~ Since other terminal carbon-oxidizing processes (e.g. denitrification, iron, and manganese reduction) outcompete sulfate reduction for electron-donating substrates, the depth of~~

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~~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 (0.1-3.9 mmol m^{-2} d^{-1}, ignoring the potentially biased value of 19.9 mmol m^{-2} d^{-1}) and the corresponding diffusive fluxes (0.01-2.4 mmol m^{-2} d^{-1}) were high compared to these published fluxes. However, 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 these latter interpretations 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 preventing significant gas formation.

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). In contrast to station B1, where an exponentially decreasing portion of sulfate reduction contributed to the total sulfate reduction at depth, at station H6 a distinct steepening in the cumulative sulfate reduction is observed below 10 cm in April, August, and October. As discussed above, we do not attribute the steepening observed in February 2013 to the same process, because sulfate was still present in abundance at this depth and methane conentrations were low and without any apparent change in gradient in this depth zone. The gradient in organoclastic sulfate reduction can be described by ~~an~~an exponential ~~law~~function (Jørgensen and Parkes, 2010),

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

where z is depth (cm) and y and b are regression coefficients (Jørgensen and Parkes, 2010). Fitting the sulfate reduction rates investigated here to such a function yielded exponential coefficients b 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 (Table 4). Since the upward flux of methane provides an additional energy source to sulfate-reducing bacteria, total 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 significant 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 raterates 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 October 2012 to 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 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 rates 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

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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 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.

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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

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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

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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

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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	**CH_4**	**CH_4**	**CH_4**	**SO_4^{2-}**	**^{35}S-SRR**	
		whole core incubation (n=4)	**whole core incubation (n=4)**	**Diffusive flux to sediment surface (n=1)**	**Diffusive flux into SMTZ (n=1)[2]**	**Diffusive flux into sediment (n=1)**	**integrated over AOM[3] zone (n=3)**	**Integrated ^{35}S-SRR (n=3)**
B1	April 2012	-19.7±2.5	-0.10±0.05	-0.1~~4~~		-0.4	no AOM zone[4]	-2.3±0.6
	August 2012	-22.5±2.9	-1.~~16~~2±0.6~~2~~	-(0.01)		-0.8	no AOM zone[4]	-0.5±0.1
	October 2012	-21.1±2.7	No data	-0.~~2~~3~~9~~		-1.4	no AOM zone[4]	-2.0±0.0.5
	February 2013	-12.0±1.5	-0.1~~09~~±0.05	-0.02		-0.2	no AOM zone[4]	-2.2±0.6
H6	April 2012	-33.5±3.5	-0.3±0.~~07~~1	-1.~~6~~7	-2.8	-2.6	(10-18 cm) = -2.8±0.7	-11.6±2.9
	April 2013		-3.9 ±0.7~~1~~[1]					
	August 2012	-26.9±2.8	-19.9±7.8~~77~~	-2.~~4~~3	-2.6	-2.7	(10-18 cm) = -2.8±0.7	-11.7±2.9
	October 2012	-25.9±2.7	-1.0~~4~~	-~~1.9~~2.0	-1.9	-2.6	(10-18 cm) = -2.4±0.6	-11.5±2.9
	February 2013	-14.9+1.6	-1.1~~2~~	-0.5~~4~~	-0.4	-1.3	no AOM zone[3]	-9.2±2.3

[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;

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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

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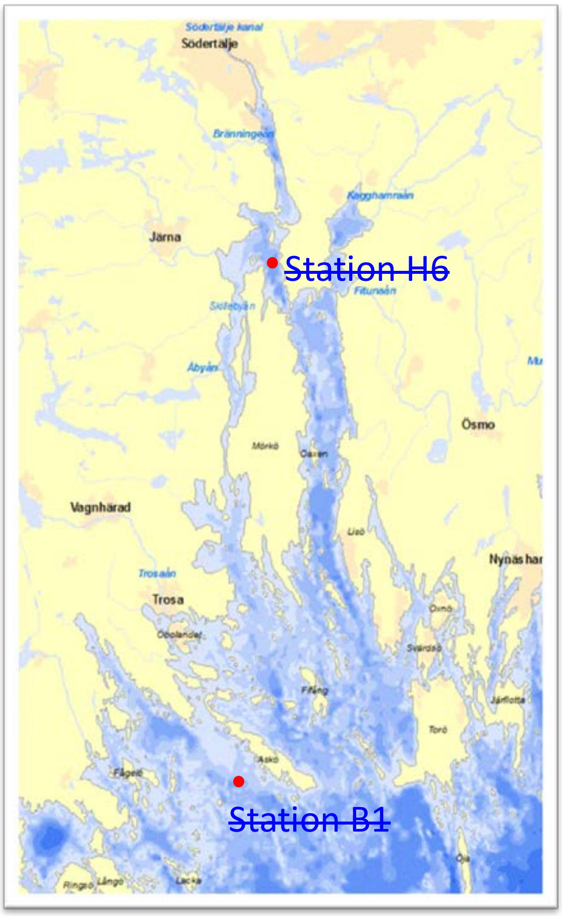
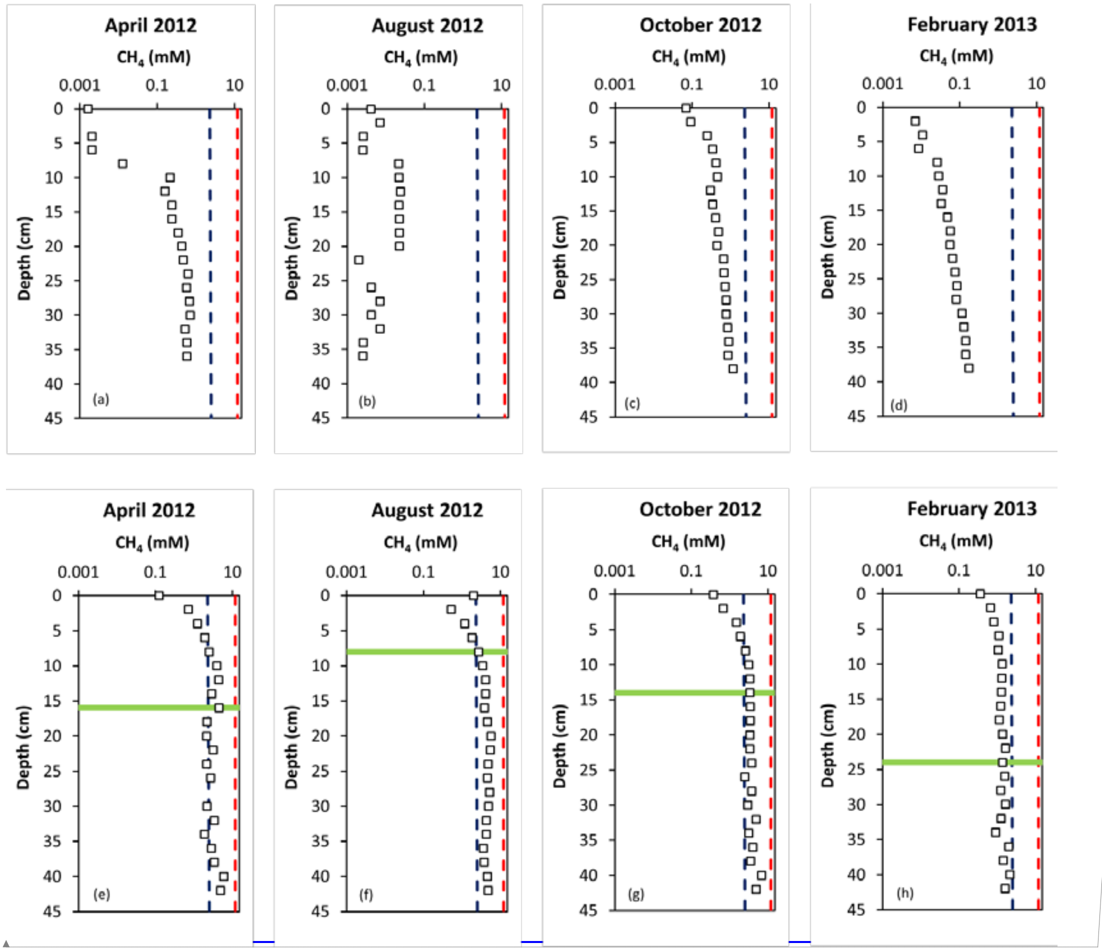


~~Figure 1. Location of sampling sites in Himmerfjärden, Stockholm Archipelago, Sweden. Detailed studies were conducted at two sites, an open water site (Station B1) and in the inner part of the estuary (Station H6).~~



~~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.~~

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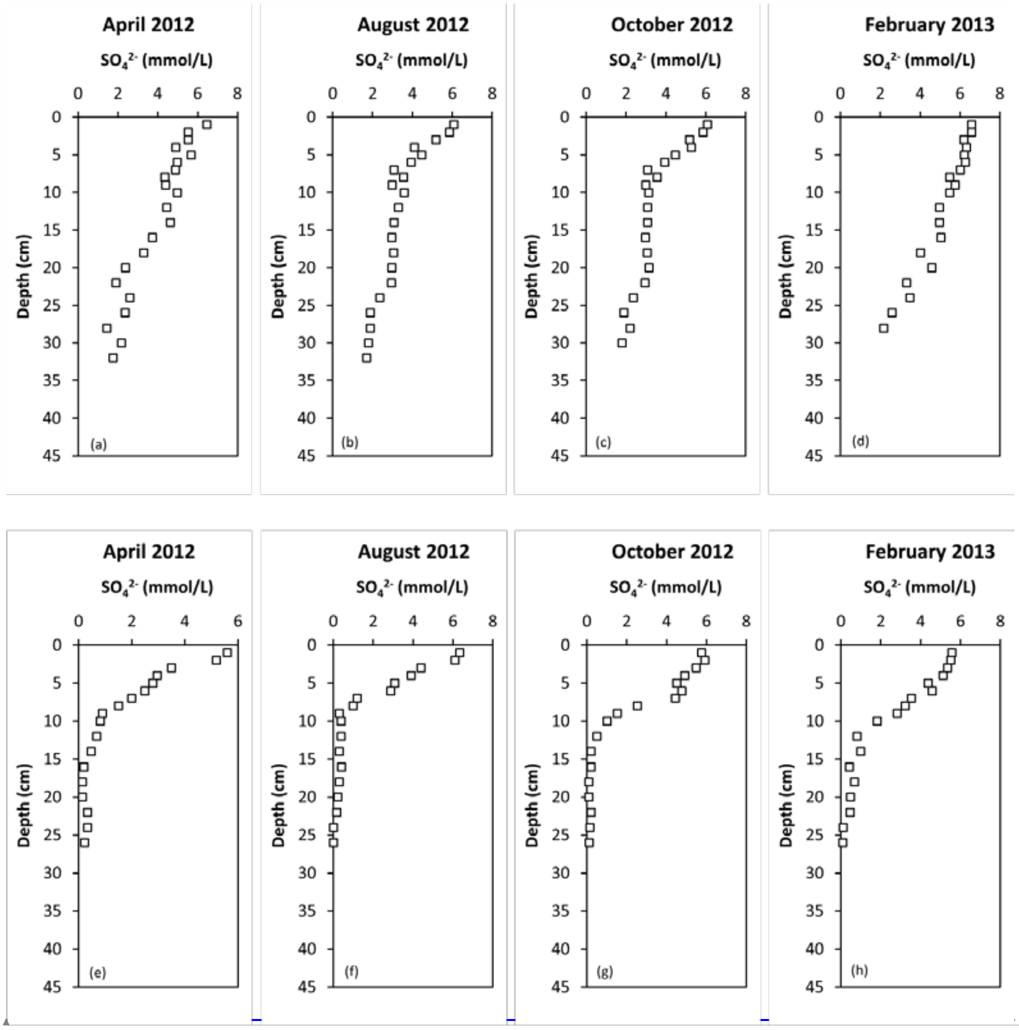

~~Figure 3. Porewater profiles of dissolved sulfate at Station B1 (a-d) and Station H6 (e-h) for the different sampling periods.~~

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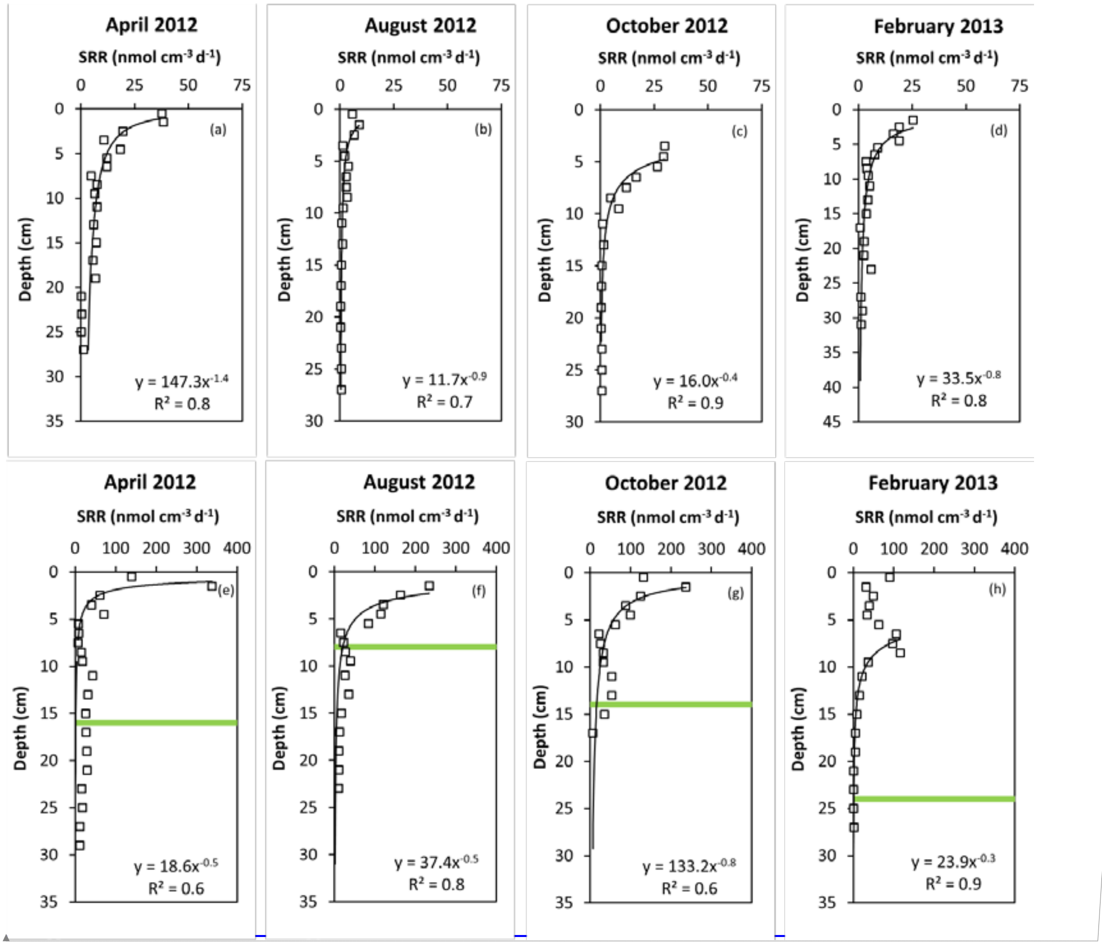

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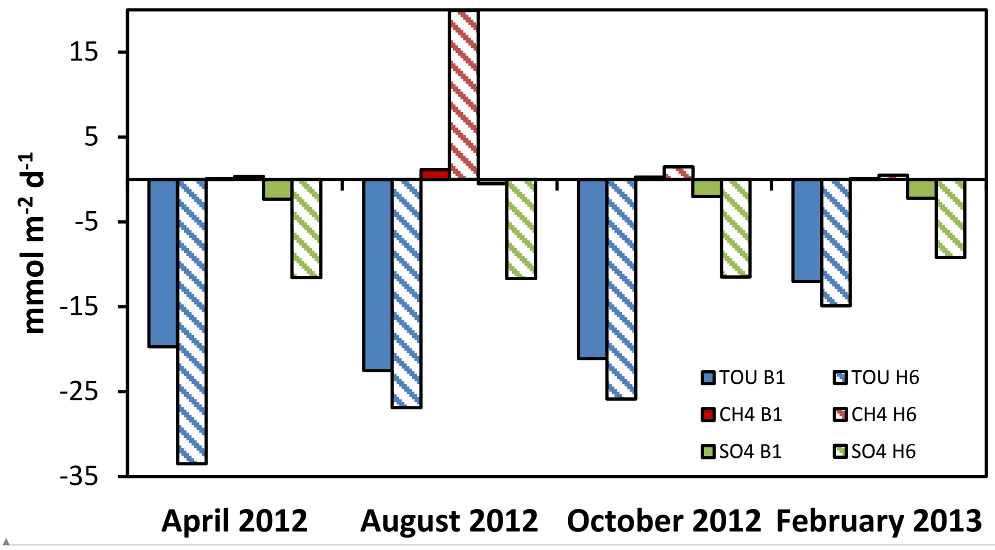
~~Figure 4. Depth gradients of bacterial sulfate reduction rates (SRR) measured with ^{35}S-sulfate. Black lines show the regression results to a power law of the form y = ax^{-b}. The green line marks the maximum depth of sulfate penetration.~~



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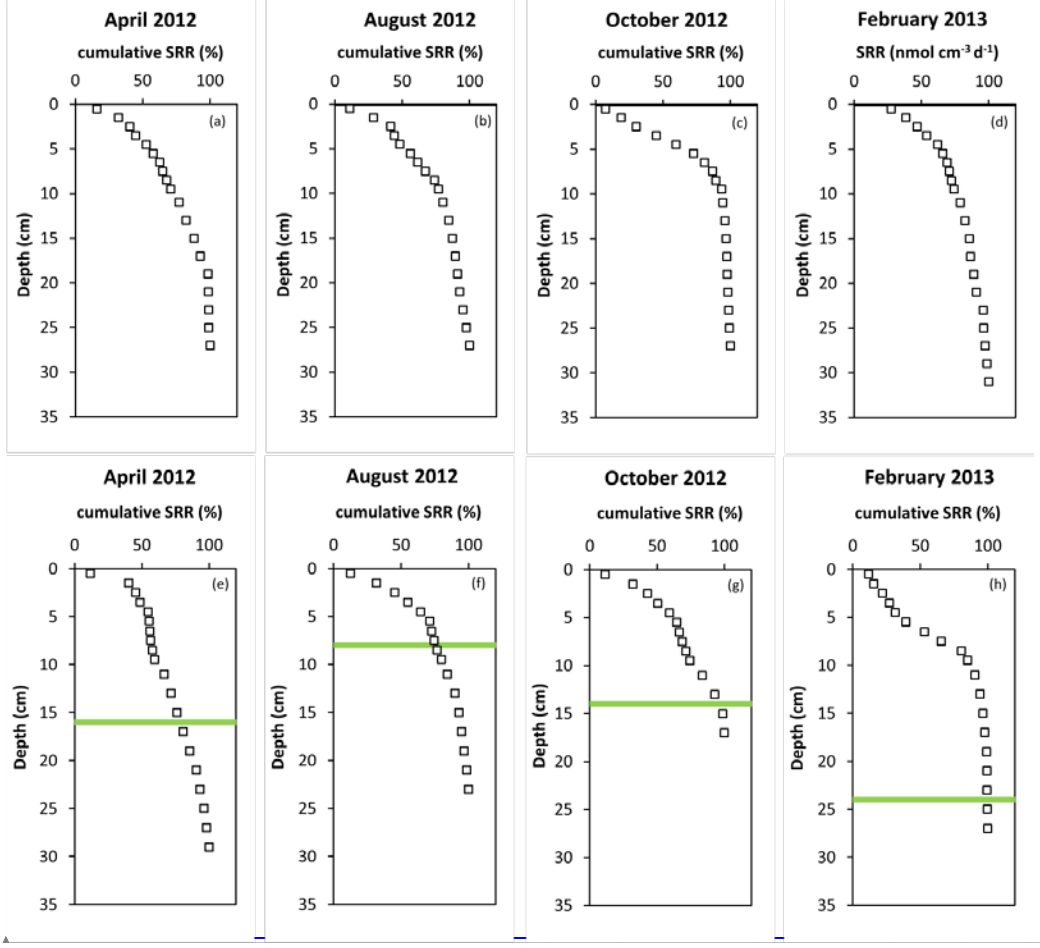
~~Figure 5. Comparison of benthic fluxes (mmol m^{-2} d^{-1}) for sulfate (SO$_4$), methane (CH$_4$), and oxygen for the different sampling periods.~~



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

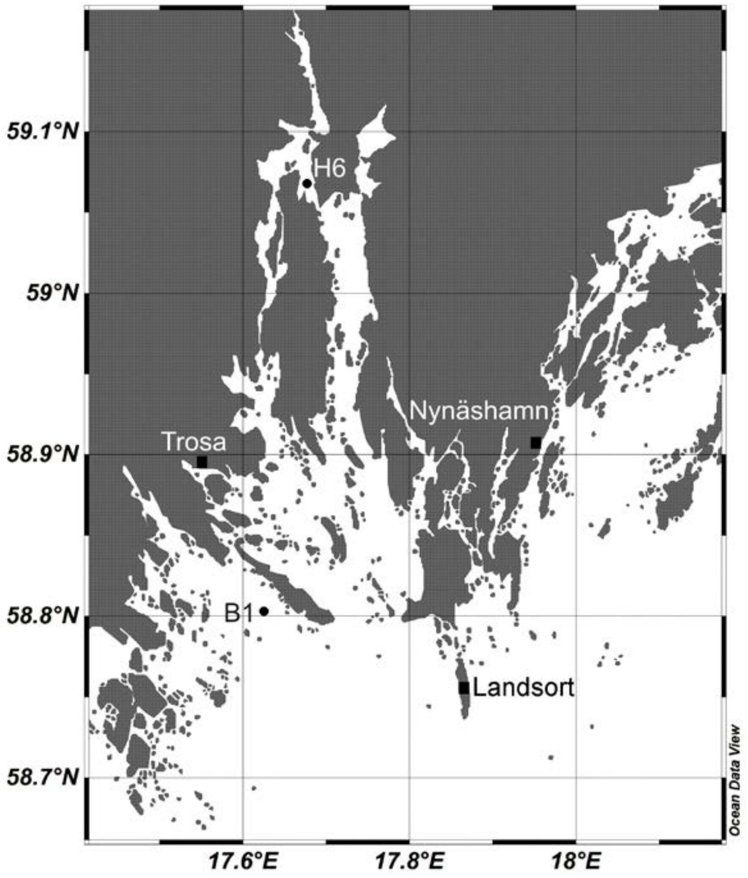

Figure 1. Location of sampling sites in Himmerfjärden, Stockholm Archipelago, Sweden. Detailed studies were conducted at two sites, an open water site (s~~S~~tation B1) and in the inner part of the estuary (s~~S~~tation H6).

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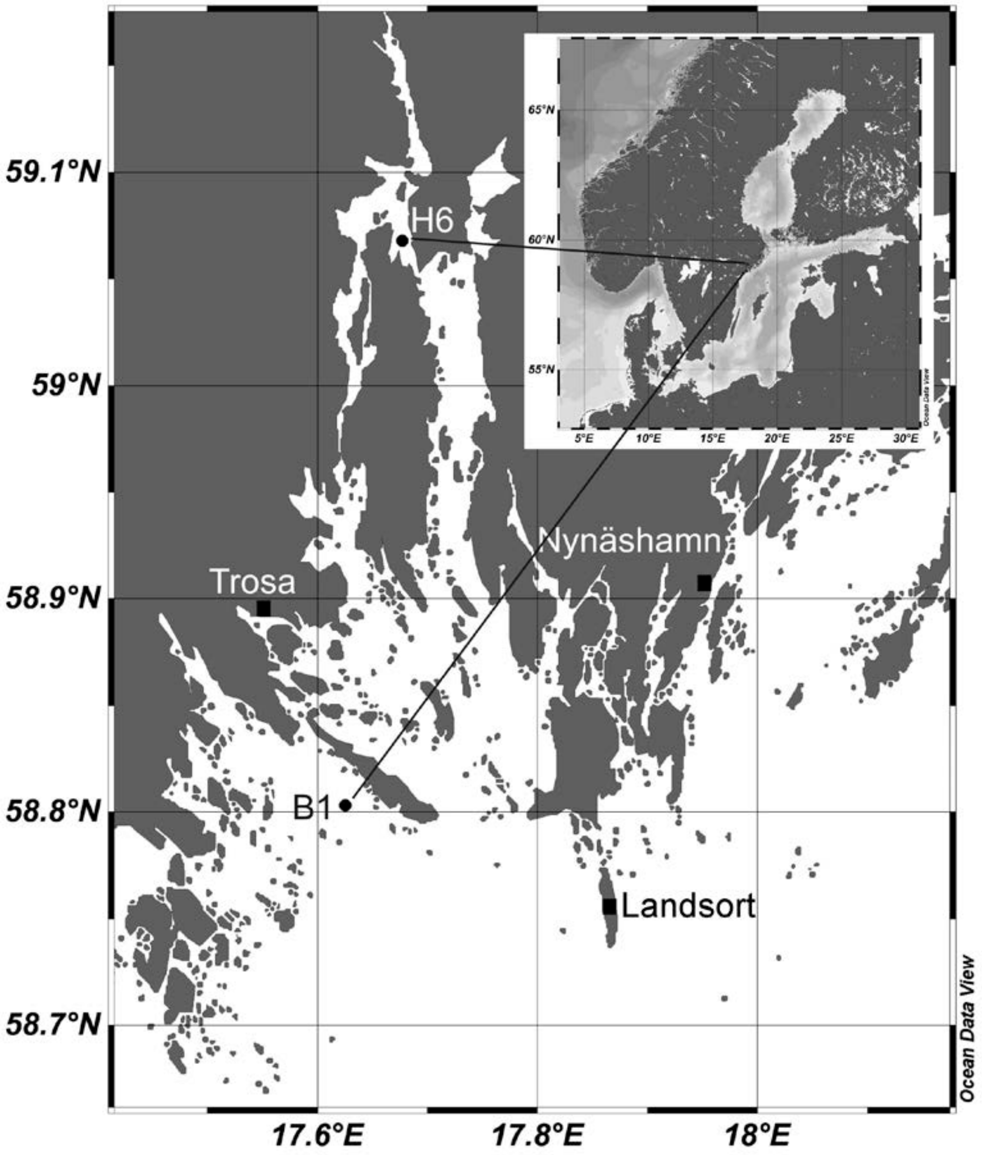
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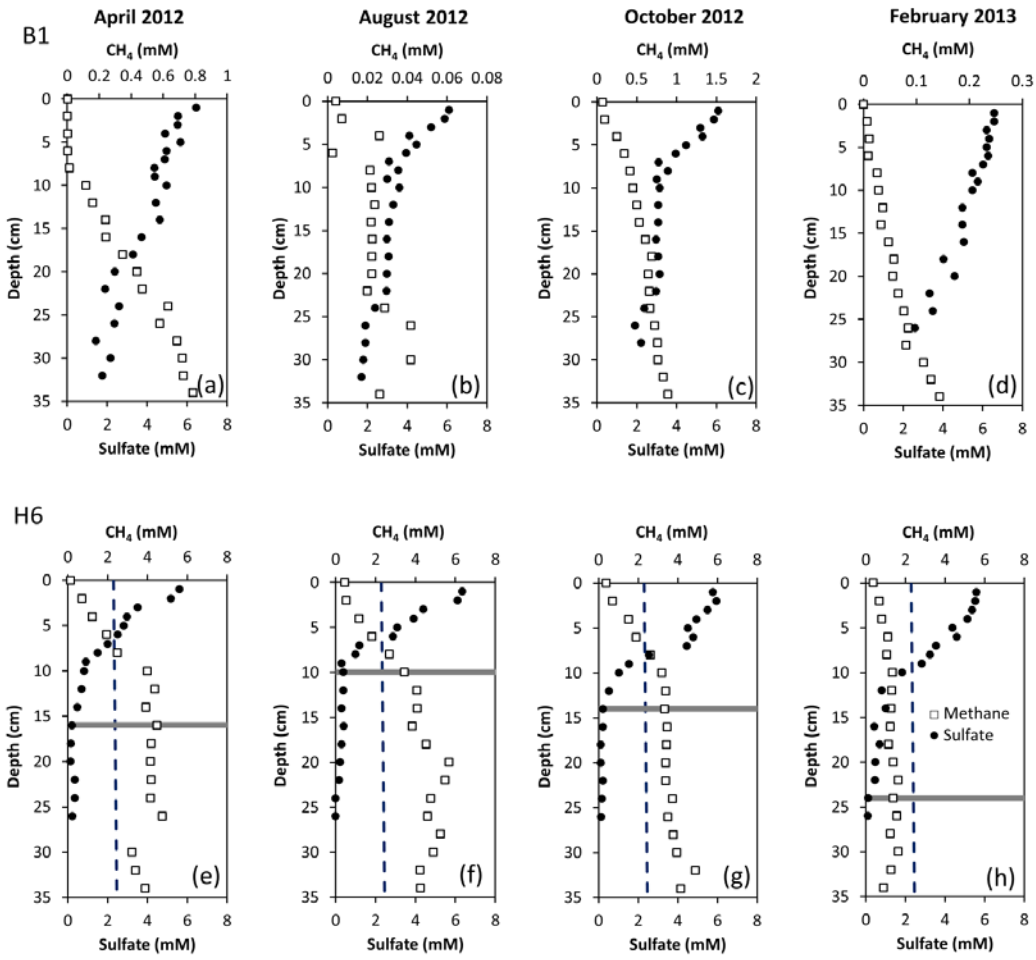


Figure 2. Porewater profiles of total methane and sulfate at Station B1 (a-d) and Station H6 (e-h) for the different sampling periods. The grey line marks the initial minimum sulfate concentration depth. Dashed lines indicate the methane saturation concentration at 1 atm pressure (grey) at the time of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

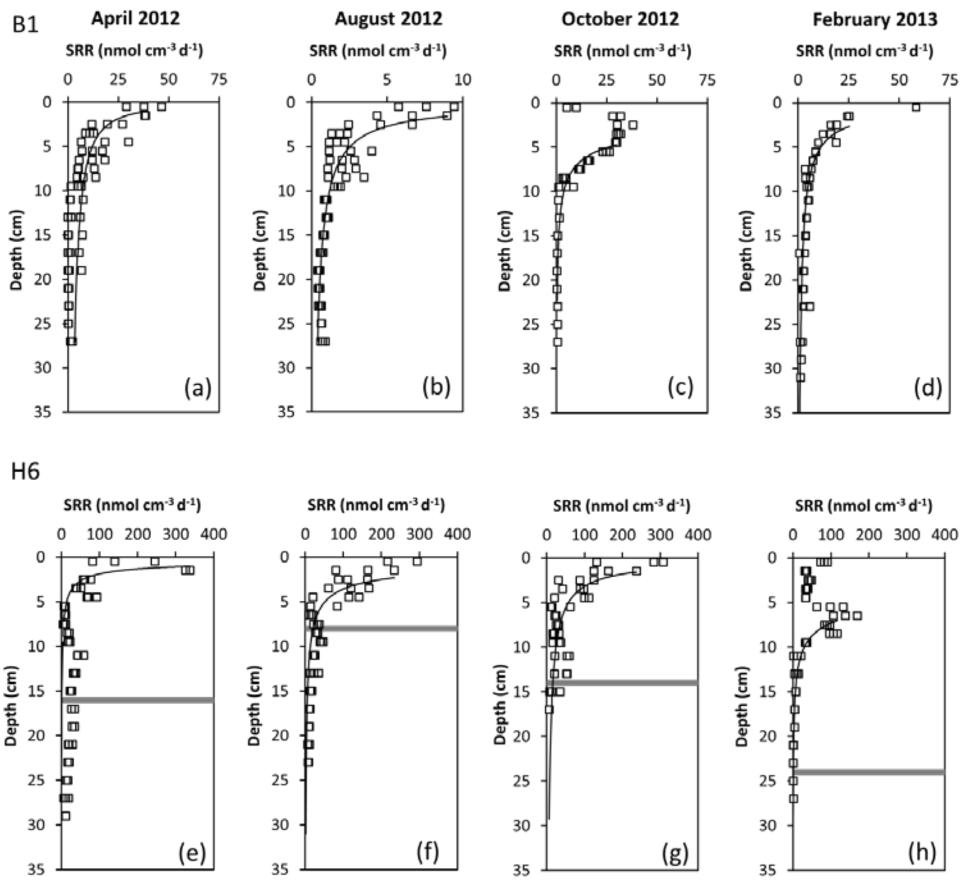


Figure 3. Depth gradients of bacterial sulfate reduction rates (SRR) measured with ^{35}S-sulfate at Station B1 (a-d) and Station H6 (e-h) for the different sampling periods. Black lines show the regression results to a power function of the form $y = ax^{-b}$. The grey line marks the initial minimum sulfate concentration depth.

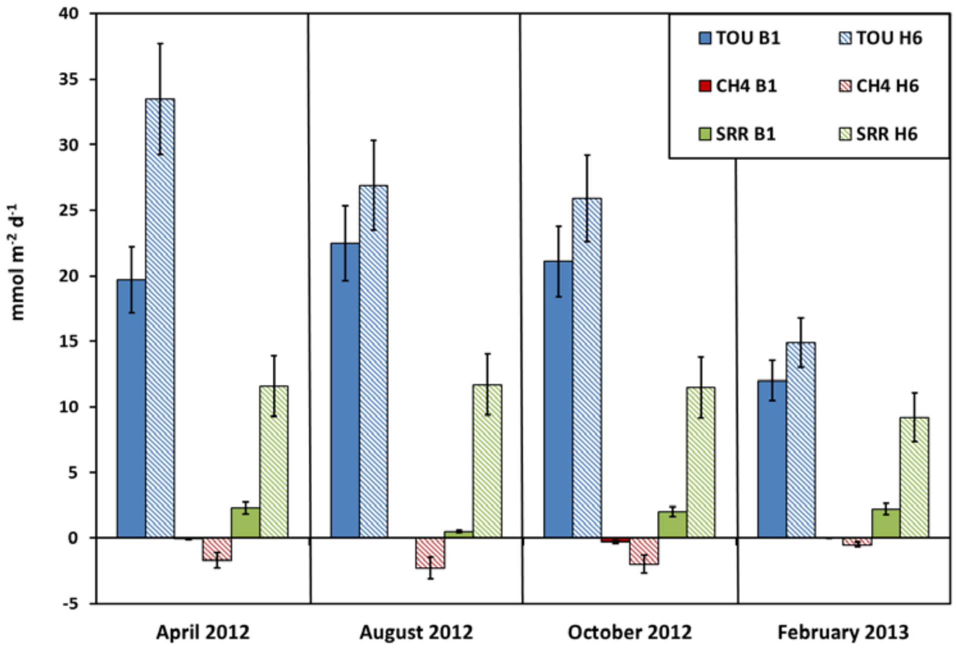
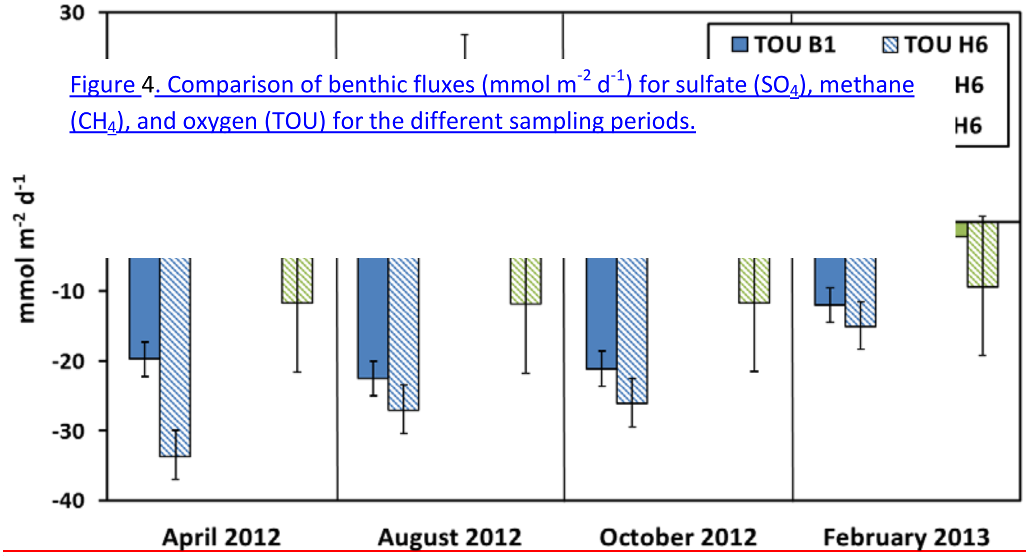


Figure 4. Comparison of benthic fluxes (mmol m^{-2} d^{-1}) for sulfate (SO_4), methane (CH_4), and oxygen (TOU) for the different sampling periods.



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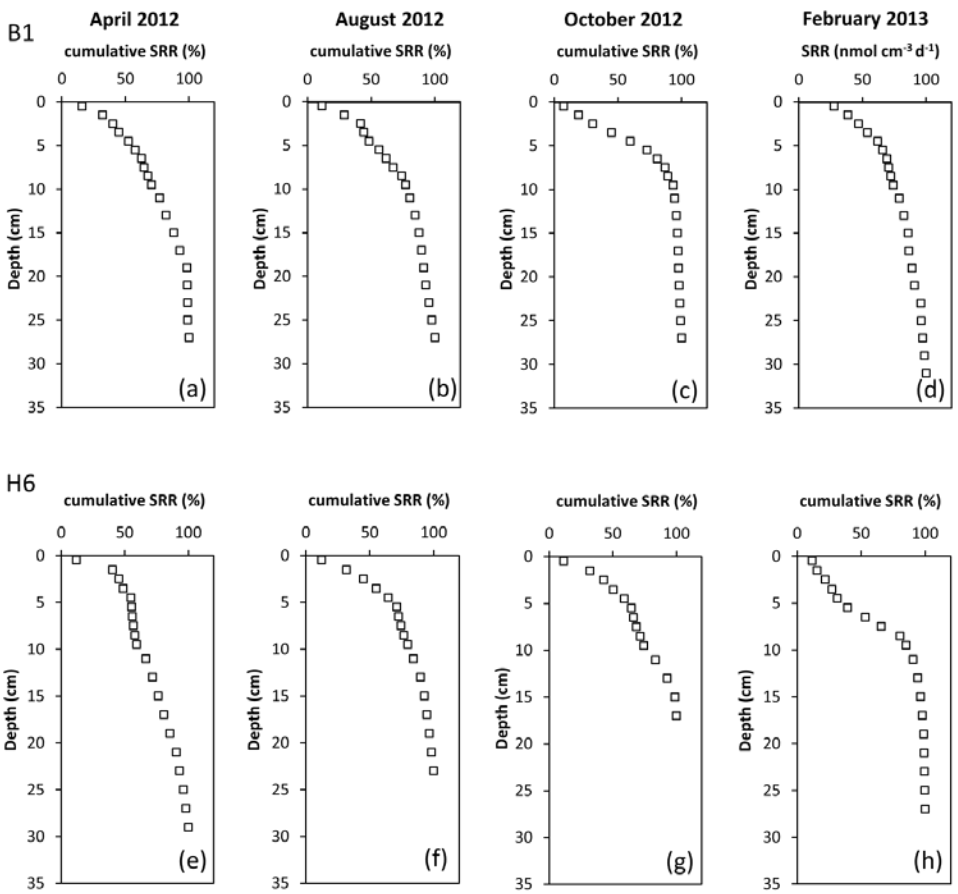

Figure 5. Depth distribution of sulfate reduction rate expressed as cumulative percentage at Station B1 (a-d) and Station H6 (e-h) for the different sampling periods. The grey line marks the initial minimum sulfate concentration depth.