

1 3rd Response to manuscript "Annual Variability and regulation of methane in sulphate fluxes in
2 Baltic Sea estuarine sediments." by Joanna E. Sawicka and Volker Bruechert

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5 To the editor:

6
7 Dear Tina Treude,

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9 We have now addressed your comments and made the recommended changes to the manuscript.
10 Please find all our changes listed below with line indications of the present document, where the
11 changes have been made.

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14 Best regards,

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16 Volker Brüchert and Joanna Sawicka

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20
21 533 (see lines : Please enter name of research vessel ("on-board" which vessel?)

22 Inserted: lines 199/200: research vessel R/V Limanda and ice class research vessel R/V Aurelia

23
24 633: Please provide a one sentence introduction of the purpose of this incubation at the beginning of the
25 paragraph.

26 Inserted: lines 275/276

27 "In order to account for the total benthic exchange of oxygen and methane by advection, diffusion, bioirrigation,
28 and bioturbation, four intact cores ..."

29
30 643 and 653: I suggest to make these sub-capters of 2.7, because they belong to the incubation (2.7.1 and 2.7.2)

31 We changed the sections accordingly 2.7 w/ intro, then 2.7.1 for O₂ uptake and 2.7.2 for **Benthic** methane fluxes

32
33 654: Methane fluxes of what? Benthic fluxes? Please add information.

34 Added **Benthic** methane fluxes

35
36 654: Was the water sample taken close to the sediment interface or in the bulk supernatant water. Please clarify.

37 added line 297: "directly above the sediment-water interface"

38
39 679: Chapter must than be 2.8 (see above)

40 Has been changed

41
42 735: Please comment that the detection limit of the IC is 100 µM for sulfate (see your comment in the answers to
43 the reviewer)

44 Added section 2.5 line 248/249: The detection limit for sulfate after necessary dilution to reduce the chloride
45 peak size was 100 µM.

46
47 744: Rates integrated over which depth?

48 Specified line 376 (B1) and line 389 (H6): "over the total core length"

49
50 865: "comparable" should better read "similar"

51 Changed in line 471

52
53 908: "Sediment focusing" sounds a bit strange. Rewording?
54 Changed to (line 512): "Preferential accumulation of sediment in the bathymetric depressions ..."
55
56 933: Sentence has 2 x "to greater depth". Maybe re-wording?
57 Changed to (line 535-537):

58 " In February, reduced organic carbon input and higher oxygen concentrations resulted in lower sulfate reduction
59 rates and a downward displacement of the maximum rates confining methane production to greater depths in the
60 sediment."

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

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90 **Abstract.** Marine methane emissions originate largely from near-shore coastal systems, but emission
91 estimates are often not based on temporally well-resolved data or sufficient understanding of the
92 variability of methane consumption and production processes in the underlying sediment. The
93 objectives of our investigation were to explore the effects of seasonal temperature, changes in benthic
94 oxygen concentration, and historical eutrophication on sediment methane concentrations and benthic
95 fluxes at two type localities for open-water coastal versus eutrophic, estuarine sediment in the Baltic
96 Sea. Benthic fluxes of methane and oxygen, sediment porewater concentrations of dissolved sulfate,
97 methane, and ³⁵S-sulfate reduction rates were obtained over a 12-month period from April 2012 to
98 April 2013. Benthic methane fluxes varied by factors of 5 and 12 at the offshore coastal site and the
99 eutrophic estuarine station, respectively, ranging from 0.1 mmol m⁻²d⁻¹ in winter at an open coastal site
100 to 2.6 mmol m⁻²d⁻¹ in late summer in the inner eutrophic estuary. Total oxygen uptake (TOU) and ³⁵S-
101 sulfate reduction rates (SRR) correlated with methane fluxes showing low rates in the winter and high
102 rates in the summer. The highest porewater methane concentrations also varied by factors of 6 and 10
103 over the sampling period with lowest values in the winter and highest values in late summer-early
104 autumn. The highest porewater methane concentrations were 5.7 mM a few centimeters below the
105 sediment surface, but never exceeded the in-situ saturation concentration. 21 – 24% of the total sulfate
106 reduction was coupled to anaerobic methane oxidation lowering methane concentrations below the
107 sediment surface far below the saturation concentration. The data imply that bubble emission likely
108 plays no or only a minor role for methane emissions in these sediments. The changes in porewater
109 methane concentrations over the observation period were too large to be explained by temporal changes
110 in methane formation and methane oxidation rates due to temperature alone. Additional factors such as
111 regional and local hydrostatic pressure changes and coastal submarine groundwater flow may also
112 affect the vertical and lateral transport of methane.

113

114 **Keywords** Methane cycling, coastal and estuarine sediment, seasonality

115 **1 Introduction**

116 The world's estuaries have been suggested to emit between 1.8 and 6.6 Tg CH₄ y⁻¹ to the atmosphere
117 (Borges and Abril, 2011; Amouroux et al 2002, Marty et al., 2001; Middelburg et al., 2002; Sansone et
118 al., 1999; Upstill-Goddard et al., 2000), a considerable portion of the estimated total oceanic emissions
119 of 10-30 Tg CH₄ y⁻¹ (Judd, 2004; Etiope et al., 2008; Kirschke et al., 2013). As other globally upscaled
120 estimates of emissions, these estimates also have considerable uncertainties. In the case of estuaries, a
121 major cause of the uncertainty are relatively few spatially and temporally resolved measurements of
122 anaerobic carbon degradation measurements in sediments and measurements of methane fluxes from
123 sediments. In estuarine waters methane can be derived from underlying anoxic sediments, transported
124 laterally due to freshwater or sewage discharge, seepage of methane-rich groundwater, or it can be
125 derived from near-shore aquatic plants (Borges and Abril, 2011). The amount of sedimentary methane
126 production in estuaries is a function of organic matter availability, bottom water oxygen concentrations,
127 and the salinity of the estuary. Methane production is generally greater in low-salinity estuaries because
128 of lower sulfate availability to promote bacterial sulfate reduction (Borges and Abril, 2011). Methane
129 fluxes from estuarine sediments are characterized by significant spatial and temporal variability
130 (Borges and Abril 2011). Temporal patterns show that concentrations and fluxes of CH₄ are generally
131 higher in the warmer season and low in the colder season (Crill et al., 1983, Martens and Klump, 1984,
132 Musenze et al., 2014; Reindl and Bolalek, 2014). Notably, very few studies have considered CH₄ fluxes
133 in high-latitude environments during snow- and ice-covered periods. While shallow systems within the
134 tidal range derive a significant amount of the methane flux from ebullition (Martens and Klump, 1984),
135 groundwater discharge, tidal pumping, and transport by aquatic plants (Middelburg et al., 2002;
136 Kristensen et al 2008), the transport from deeper systems such as fjords and fjärds is thought to occur
137 largely by molecular diffusion (Abril and Iversen, 2002, Sansone et al., 1998).

138 Globally more than 90% of methane produced in marine sediments is estimated to be oxidized by the
139 anaerobic oxidation of methane (AOM), mostly in the sulfate-methane transition zone (Knittel and

140 Boetius, 2009, Martens and Berner, 1974; Jørgensen and Parkes, 2010). It is not known how much
141 methane is oxidized by AOM in estuarine sediments. In addition, up to 90% of the remaining methane
142 that reaches the sediment surface may be oxidized aerobically at the sediment surface or in the water
143 column (Reeburgh, 2007). Yet, methane concentrations in estuarine waters are almost always higher
144 than the atmospheric equilibrium concentration indicating that microbial oxidation processes and
145 physical exchange with the atmosphere in estuaries are relatively inefficient in removing methane.
146 Despite its obvious importance, only few studies have specifically addressed anaerobic oxidation of
147 methane by sulfate and aerobic oxidation in estuarine environments (e.g., Treude et al., 2005, Thang et
148 al., 2013).

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

159

160 **2 Materials and methods**

161 **2.1 Site description**

162 Himmerfjärden (Figure 1) is a fjord-type estuary with a surface area of 174 km², a volume of
163 2968 x 10⁶ m³, and a N-S bottom water salinity gradient increasing from 5.5‰ in the inner part to
164 7.0‰ at the opening to the Baltic. It is morphologically characterized by four basins, divided by sills

165 and has a low flushing rate of about 0.025/day (Savage and Elmgren, 2010). The freshwater discharge
166 is small compared to the exchange with the open Baltic and was estimated to be 23 m³/s on average in
167 2012 comprising land run-off and precipitation (30% and 21%, respectively), outflow from Lake
168 Mälaren from the north (19%) and the river Trosaån (23%), and discharge from a sewage treatment
169 plant (6%) (Larsson et al., 2012). The sewage treatment plant, built in the early 1970s, treats sewage
170 water from ca. 314,000 inhabitants of the southern Stockholm metropolitan area, and its inorganic
171 effluent is discharged mainly in the form of inorganic nitrogen and phosphorus to the inner basins
172 (Savage and Elmgren, 2010). In 2012, the sewage treatment contributed 45% of the total phosphorus
173 and 57% of the total inorganic nitrogen discharge to the northern Himmerfjärden area (Larsson et al.,
174 2012) and discharged 1676 tons carbon (measured as chemical oxygen demand COD) (Stridh, 2012).
175 The estuary undergoes thermohaline stratification during late summer and autumn, especially in the
176 inner part, which experiences regular seasonal bottom water hypoxia. The tidal range is low (few cm)
177 and relatively cold bottom waters (1.5 - 9°C) dominate throughout the year. Water level can vary
178 annually by about 50 cm depending on local wind and hydrographic conditions. Late-summer-early fall
179 bottom water hypoxia has also been reported occasionally for the outer basins of the estuary, when
180 winds are weak and circulation is inhibited (Elmgren and Larsson, 1997). Sedimentation areas in
181 Himmerfjärden can be divided into accumulation and transport bottoms (Jonsson et al., 2003). About
182 21% of the sediment surface in Himmerfjärden is classified as accumulation bottoms of particulate
183 material and receives 3.3-9 mol C m⁻² y⁻¹ (Thang et al., 2013; Karlsson et al., 2010).

184 Bottom water and sediment samples were taken from a station in the inner part of
185 Himmerfjärden, Station H6, and from a station located outside the estuary, Station B1 (Figure 1).
186 Samples were collected in April 2012, August 2012, and October 2012 with the research vessel R/V
187 Limanda, and in February 2013 with the ice-breaking research vessel R/V Aurelia. In addition, in April
188 2013 whole-core incubations were performed to determine methane and oxygen fluxes to record a full
189 year of seasonal variability. Station B1 has soft, olive grey, muddy sediment with a 1-2 cm-thick rusty
190 brown surface layer that was present year round, while the sediment at station H6 is soft, laminated

191 black mud with a 1-2 mm thin brown surface layer that occurred only during the winter and spring.
192 Sediment accumulation rates range from 0.98 cm yr⁻¹ in the innermost part of the estuary to 0.77 cm yr⁻¹
193 in the outer part of the estuary (Thang et al., 2013).

194

195 **2.2 Sample collection**

196 Sediments with well-preserved sediment surfaces were collected with a Multicorer in acrylic tubes (9.5
197 cm diameter) to 40 cm depth to determine ³⁵S-sulfate reduction rates, porosity, and the porewater
198 constituents methane and sulfate. Additional cores were collected for sediment core incubations.
199 Porewater methane samples were immediately collected on-board the research vessels R/V Limanda
200 and the ice-class vessel R/V Aurelia from the cores as described below. The other cores were capped
201 with rubber stoppers, transported to the marine laboratory on the island of Askö within 90 minutes and
202 kept cold at bottom water temperature for later experiments and subsampling. In February 2013, ice
203 partially covered Station B1 and there was complete ice cover at Station H6, and sampling was only
204 possible after ice breaking. For whole-core incubations, 30 l of bottom water was collected with a 5
205 liter HydroBios bottle and kept cold until for the experiments. Temperature, salinity, and oxygen
206 concentrations were determined with a handheld WTW Oxygen meter directly in the water overlying
207 the sediment cores.

208

209 **2.3 Organic carbon concentrations and porosity**

210 Concentrations of organic carbon were determined for the topmost cm of sediment on freeze-dried
211 sediment with a Fisons CHN elemental analyzer after treatment with 1M HCl to remove inorganic
212 carbon. Water content (%) was determined by drying 5 ml of sediment at 105°C and calculating the
213 percent loss after drying.

214

215 2.4 Methane analysis

216 Samples for methane were collected directly through the side of taped, pre-drilled core liners and taken
217 in 2-cm intervals minutes after the core was retrieved on deck. The core sampling method used in this
218 study permits complete sampling and preservation of porewater methane within 5 minutes after the
219 core was on deck. Under these circumstances, loss of methane due to gas loss was low and methane
220 concentrations could be determined for porewaters that were far above the saturation limit at 1
221 atmosphere pressure for the salinity and temperature range of the bottom water (between 1.9 mM and
222 2.4 mM). A sediment sample of 2.5 mL was taken with a 3 mL cut-off syringe. The sample was
223 transferred to a 20 mL serum vial containing 5 mL 5 M NaCl and immediately closed with a thick
224 septum and an aluminum crimp seal (Thang et al., 2013). For analysis, the sample was shaken and 5
225 mL of brine was injected into a sample vial to displace 5 mL gas out of a vial into the syringe. The CH₄
226 measurements were carried out on a gas chromatograph (GC) with a flame ionization detector (FID)
227 (SRI 8610C) after separation on a 3 feet Porapak Q pre-column before a 9 feet Hayesep D column with
228 N₂ as carrier gas. CH₄ standards 100 ppm, 1000ppm, and 10000 ppm (Air Liquide) were used for
229 calibration.

230 The concentration of methane (mM) of a sample was calculated as follows:

$$231 \quad CH_4(mM) = \frac{CH_{4\ hsp} \cdot V_{hsp}}{1000 \cdot 24.148 \cdot V_{sed} \cdot \rho} \quad (1)$$

232
233 where $CH_{4\ hsp}$ is the concentration of methane in the headspace of the sample vial (ppm), V_{hsp} is the
234 volume of the headspace (L), V_{sed} is the volume of the sediment sample (L), ρ is sediment porosity, and
235 24.148 (L mol⁻¹) is the molar volume of gas at standard pressure 100 kPa and 298 K. The
236 reproducibility of the method has been tested at a station in the archipelago that is not part of this study
237 by replicating methane sampling on multiple sediment cores. Concentrations in multiple cores deviated
238 by about 15%.

239

240 **2.5 Sulfate concentration**

241 Porewater samples for sulfate concentration measurements were obtained using rhizones (Atlas
242 Copco Welltech) (Seeberg-Elverfeldt et al 2005). Rhizones were treated for 2 hours in 2M HCl,
243 followed by two rinses with deionized water for 2 hours and final storage in deionized water. Rhizones
244 were connected to 10 mL disposable plastic syringes via 3-way luerlock stopcocks and inserted in 1-cm
245 intervals through tight-fitting, pre-drilled holes in the liner of the sediment cores. The first mL of pore
246 water was discarded from the syringe. No more than 2 ml were collected from each core to prevent
247 cross-contamination of adjacent intervals (Seeberg-Elverfeldt et al., 2005). Sulfate concentrations were
248 determined with a Dionex System IC 20 ion chromatograph. The detection limit for sulfate after
249 twentyfold dilution to reduce the chloride peak size was 100 μM .

250

251 **2.6 ^{35}S -Sulfate reduction rates**

252 To determine bacterial sulfate reduction rates (SRR) sediment cores were subsampled in 40-cm
253 long 28 mm-diameter cores with 1-cm spaced, silicon-sealed, pre-drilled small holes on the side for
254 injections. For the incubation, the whole-core incubation method by Jørgensen (1978) was used. $^{35}\text{SO}_4^{2-}$
255 tracer solution was diluted in a 6 ‰ NaCl solution containing 0.5 mM SO_4^{2-} . 2.5 μl of the tracer
256 solution (50kBq) was injected through the pre-drilled holes. The cores were then capped and sealed in
257 plastic wrap foil and incubated for 8 hours at the respective bottom water temperatures. After this time,
258 the incubations were stopped by sectioning the core in 1-cm intervals to 5 cm depth and in two
259 centimeter intervals below this depth to the bottom of the core. Sediment sections were transferred to
260 50 ml plastic centrifuge tubes containing 20 ml zinc acetate (20% v/v) and shaken vigorously and
261 frozen. The total amount of ^{35}S -labeled reduced sulfur (TRIS) was determined using the single-step
262 cold chromium distillation method by Kallmeyer et al. (2004). TRIS and supernatant sulfate were
263 counted on a TriCarb 2095 Perkin Elmer scintillation counter. The sulfate reduction rate was calculated
264 using the following equation (Jørgensen, 1978):

265
$${}^{35}\text{SRR} = \left(\text{TRI}^{35}\text{S} / ({}^{35}\text{SO}_4^{2-} + \text{TRI}^{35}\text{S}) \right) \cdot 1.06 \cdot \text{SO}_4^{2-} \cdot \rho \cdot 1/t \quad (2)$$

266 where SO_4^{2-} is the pore water sulfate concentration corrected for porosity ρ , TRI^{35}S and ${}^{35}\text{SO}_4^{2-}$ are the
267 measured counts (cpm) of total reduced inorganic sulfur species and sulfate, respectively, 1.06 is a
268 correction factor accounting for the isotope discrimination of ${}^{35}\text{S}$ against ${}^{32}\text{S}$ -sulfate, and t is the
269 incubation time. The sulfate reduction rate is reported as $\text{nmol cm}^{-3} \text{ day}^{-1}$. Generally, when enough
270 cores were available ${}^{35}\text{SRR}$ were measured on replicate cores for all depth intervals. The detection limit
271 of the rate measurements accounting for distillation blanks and radioactive decay of ${}^{35}\text{S}$ between
272 experiment and laboratory workup was $0.1 \text{ nmol cm}^{-3} \text{ day}^{-1}$.

273

274 **2.7 Whole-core sediment incubations**

275 In order to account for the total benthic exchange of oxygen and methane by advection, diffusion,
276 bioirrigation, and bioturbation, four intact cores with undisturbed sediment surfaces and clear overlying
277 water were subsampled in the laboratory in acrylic tubes (i.d. 6.2 cm, height 25 cm) retaining about 10
278 cm of the overlying water. The sediment height in the tubes was approximately 10 cm. The cores were
279 incubated in a 40-liter incubation tank filled with bottom water from the same station. Before the
280 incubation the overlying water in the cores was equilibrated with bottom water in the tank. The
281 overlying water in the cores was stirred by small magnetic bars mounted in the core liners and driven
282 by an external magnet at 60 rpm. The cores were pre-incubated uncapped for 6 hours and subsequently
283 capped and incubated for a period of 6 to 12 hours depending on the initial oxygen concentration in the
284 bottom water.

285

286 **2.7.1 Total oxygen uptake**

287 Oxygen sensor spots (Firesting oxygen optode, PyroScience GmbH, Germany) with a sensing surface
288 of 5 mm diameter were attached to the inner wall of two incubation cores (diameter 5.5 cm). The
289 sensor spots were calibrated against O₂-saturated bottom water and oxygen-free water following the
290 manufacturer's guidelines accounting for temperature and salinity of the incubation water.
291 Measurements were performed with a fiberoptic cable connected to a spot adapter fixed at the outer
292 core liner wall at the spot position. The O₂ concentration was continuously logged during incubations.
293 Sediment total oxygen uptake (TOU) rates were computed by linear regression of the O₂ concentration
294 over time.

295

296 **2.7.2 Benthic methane fluxes**

297 Benthic methane fluxes were determined from discrete water samples directly above the sediment-
298 water interface and collected without headspace in 12 mL Exetainers (Labco, Wycombe, UK) prefilled
299 with 50 µL of 50% ZnCl₂. Samples were collected at the beginning (time zero) and the end of the
300 incubation (time final), usually after 24 hours. CH₄ concentrations were determined using the
301 headspace equilibration technique (Kampbell et al., 1989) by replacing 3 ml of the water in the
302 exetainers with high-purity helium gas at atmospheric pressure. The Exetainers were then shaken at
303 400rpm on a shaking table for 60 minutes to allow the gas to equilibrate between the headspace and the
304 liquid phase and left to rest for half an hour. After equilibration 2.5 mL of NaCl brine was injected into
305 an Exetainer to force the gas samples into an injection syringe while maintaining the headspace
306 pressure. The samples were injected onto a 1 ml injection loop of a gas chromatograph (SRI 8610C)
307 with FID detector using N₂ as carrier gas. CH₄ standards 5 ppm, 100 ppm and 1000 ppm (Air Liquide)
308 were used to construct a calibration curve. The partial pressure of CH₄ in the equilibrated headspace
309 and water was calculated using the solubility coefficient β for CH₄ using the salinity of the bottom
310 water at the respective sample time (Table 1) (Wilhelm et al 1977), gas constant R (8.314 L kPa mol⁻¹)

311 K^{-1}), air pressure P (kPa), headspace gas concentration $CH_{4\text{ hsp}}$ (nmol), headspace volume (0.003L),
312 water volume in the exetainer (0.009L), and laboratory temperature T (293 K) according to

$$313 \quad CH_4 \text{ (nM)} = (CH_{4\text{ hsp}} + \beta CH_{4\text{ hsp}}) * P/RT \quad (3)$$

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

$$316 \quad J = (CH_{4\text{ start}} - CH_{4\text{ end}})/t * V/A \quad (4)$$

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

319

320 **2.8 Diffusive flux calculations**

321 Diffusive fluxes of methane and sulfate were estimated from the porewater gradients of methane and
322 sulfate for the sediment surface and the sulfate-methane transition zone. Sediment cores at station B1
323 showed occasional burrows from deposit feeders in the topmost 2 cm of sediment, whereas sediment at
324 station H6 was largely devoid of macro- and meiofauna. Since only one sample was taken from the
325 topmost 2 cm, quantitative depth-related effects of bioturbation cannot be accounted for in this analysis
326 and upward diffusive transport of methane was assumed as the dominant transport pathway. Fluxes
327 were estimated using Fick's first law of diffusion

$$328 \quad J = D_s \frac{dC}{dx} \quad (5)$$

329 assuming that flux was dominated by molecular diffusion, where dC is the change in concentration of
330 dissolved sulfate (mM) or methane (mM) over a depth interval dx (cm), and D_s is the sediment
331 diffusion coefficient calculated for the bottom water temperature and salinity according to Boudreau
332 (1996). D_s was recalculated from the molecular diffusion coefficient D_o for sulfate and methane
333 according to Iversen and Jørgensen (1994). Since the resolution of the porewater methane analysis was
334 2 cm, concentration changes below this resolution cannot be resolved. This could lead to an

335 overestimation of the flux across the sediment surface, e.g., due to aerobic methane oxidation in the
336 topmost mm of sediment. Similar effects may occur in the sulfate-methane transition zone.

337

338 **3. Results**

339 **3.1 Bottom water temperature, dissolved oxygen, sediment organic carbon**

340 Over the observation period April 2012 through February 2013 bottom water salinity varied between
341 6.5 and 7.0‰ at station B1 and 5.4 and 6.5‰ at Station H6 (Table 1), while bottom water temperatures
342 ranged from 2.4°C to 6.9°C for station B1 and 1.8°C to 9.4°C for station H6. The lowest and highest
343 bottom water oxygen concentrations measured were 160 µM for station B1 and 40 µM for station H6 in
344 April 2012, and 300 µM and 380 µM for station B1 and station H6 in February 2013, respectively.
345 Surface sediment organic carbon concentrations were similar at the two stations ranging between 4.6
346 and 5.2% at Station B1, and 5.0% and 6.0% at Station H6 over the observation period.

347

348 **3.2 Methane and sulfate concentrations**

349 At both stations, the measured methane concentrations never exceeded the solubility limit for methane
350 calculated for the *in situ* pressure, which ranged from 9.6 to 11.9 mM during the different sampling
351 periods. At station B1, the highest methane concentrations in the sediment cores were recorded in
352 October 2012, when they reached 0.9 mM (Figure 2a-d). Surprisingly, the lowest methane
353 concentrations were recorded in August 2012. This was possibly due to drift of the vessel during
354 sampling in rough seas at that time into an area underlain by neighbouring glacial clays with low
355 porewater methane concentrations. Excluding the August data, methane concentrations were low and
356 between 1 and 10 µM to a depth of 6 cm, 2 cm, and 6 cm in April, October, and February, respectively,
357 before they increased sharply. At station H6, the highest and lowest concentrations in the cored depth
358 interval were 5.7 mM and 1.5 mM, and recorded in August and February 2013, respectively. At this

359 station, the methane concentrations generally increased linearly from the surface down to 10 cm depth.
360 Below this depth they only increased slightly or remained constant.
361 Sulfate concentration gradients changed between the different seasons at both stations reflecting
362 changes in sulfate reduction rates over the observation period. At both stations, the sulfate
363 concentration gradients were steepest in the topmost 8-10 cm in August, intermediate in April and
364 October, and lowest in February indicating highest and lowest sulfate reduction rates in late summer
365 and winter, respectively (Figure 2 a-h). At station B1, sulfate was never consumed completely and
366 concentrations remained above 1.5 mM at the bottom of the core. In August and October, a distinct
367 decrease in the sulfate concentration gradient occurred at around 8-10 cm depth. Despite some
368 variability in the sulfate concentration profiles, the sulfate concentrations at the bottom of the core were
369 similar during all observation periods. At station H6, sulfate always reached minimum detection
370 concentrations of less than 100 μM in the cored sediment interval, albeit at substantially greater depth
371 in February. The initial depth at which sulfate reached the lowest concentration from the surface down
372 was defined as the initial minimum sulfate concentration depth, which occurred at 16 cm depth in
373 April, 10 cm in August, 14 cm in October and at 25 cm depth in February.

374

375 **3.3 ^{35}S -sulfate reduction rates**

376 At Station B1, the depth-integrated SRR over the total core length varied from 0.5 to 2.3 $\text{mmol m}^2 \text{d}^{-1}$.
377 The depth-resolved SRR ranged from 63 $\text{nmol cm}^{-3} \text{d}^{-1}$ at the sediment surface to 0.2 $\text{nmol cm}^{-3} \text{d}^{-1}$ at
378 the bottom of the cored intervals (Figure 3 a-h, Table 2). Contrary to expectations, the lowest SRR
379 were measured in August, which was possibly also due to the fact that the vessel drifted into a glacial
380 clay area. The highest SRR were measured in the topmost 2 cm with the exception of October 2012,
381 when the maximum was found at 3 cm depth. Below the depth of maximum SRR, rates decreased
382 exponentially indicating that organoclastic sulfate reduction dominated and that the reactivity of the
383 degrading organic material decreased exponentially with depth. More than 90% of the integrated
384 sulfate reduction took place in the top 15 cm of sediment (Figure 5 a-d). Over the cored sediment

385 interval, there was no peak that could be attributed to significant AOM. Nevertheless, the distinct
386 curvature of the methane concentration profile in February 2013 at station B1 suggests that methane
387 was oxidized in the sulfate reduction zone and that some of the sulfate reduction may have been
388 coupled to anaerobic methane oxidation.

389 At Station H6, depth-integrated SRR over the total core length varied from 9.2 to 11.7 mmol m⁻² d⁻¹.
390 The highest measured SRR was 338 nmol cm⁻³ d⁻¹ and occurred at 2 cm depth in April 2012.

391 Organoclastic sulfate reduction dominated the interval down to 10 cm. In April, August, and October
392 2012 two distinct sulfate reduction rate peaks were found at station H6, one at the surface, and a second
393 peak between 10 cm and 18 cm depth. The latter peak covers the sulfate-methane transition zone and
394 indicates that in this depth interval the rates of anaerobic methane oxidation coupled to sulfate
395 reduction exceeded organoclastic sulfate reduction rates. We therefore defined the depth interval near
396 the minimum sulfate concentration depth together with elevated SRR as the AOM zone (Table 2).

397 Previous studies at nearby station H5 in Himmerfjärden also found AOM to be present at depths
398 between 6 and 16 cm, which is in agreement with our findings (Thang et al., 2013; Wegener et al.,
399 2012). The depth-integrated rates of SRR in the sulfate-methane transition zone at H6 were relatively
400 constant over the three observation periods and varied between 2.4 mmol m⁻² d⁻¹ and 2.8 mmol m⁻² d⁻¹
401 (Table 2). In February, however, when sulfate penetrated to 24 cm depth, sulfate reduction rates were
402 about two times lower compared to the other months. The previously observed elevated rates between
403 10 and 18 cm depth were not visible, although another SRR peak was observed between 5 and 9 cm
404 depth. However, the high concentrations of sulfate and low concentrations of methane in this depth
405 interval in February make it unlikely that this peak is due to AOM. It is more likely that this peak is
406 associated with organoclastic sulfate reduction, because no change in the sulfate or methane gradients
407 was observed at this depth. Some sulfate reduction was also detected below 18 cm depth at station H6
408 in April, August, and October. Since non-radioactive carrier sulfate was added to the ³⁵S-tracer during
409 these incubations, these rates indicate potential sulfate reduction activity in the methanogenic zone
410 (Leloup et al., 2009).

411

412 **3.4 Benthic exchange of oxygen, sulfate, and methane**

413 Rates of total oxygen uptake are summarized in Table 2 and shown for comparison in Figure 4. Total
414 oxygen uptake was lowest in February at both stations (B1: $12.0 \pm 1.5 \text{ mmol m}^{-2} \text{ d}^{-1}$ and H6: 14.9 ± 1.6
415 $\text{mmol m}^{-2} \text{ d}^{-1}$), and highest in August at station B1 ($22.5 \pm 2.9 \text{ mmol m}^{-2} \text{ d}^{-1}$) and in April at station H6
416 ($33.5 \pm 3.5 \text{ mmol m}^{-2} \text{ d}^{-1}$). Diffusive fluxes of sulfate from the water column into the sediment ranged
417 from $0.2 \text{ mmol m}^{-2} \text{ d}^{-1}$ in February to $1.4 \text{ mmol m}^{-2} \text{ d}^{-1}$ in October at station B1, and from 1.3 mmol m^{-2}
418 d^{-1} in February to $2.7 \text{ mmol m}^{-2} \text{ d}^{-1}$ in August at station H6 (Table 2). These rates are significantly
419 lower than the depth-integrated radiotracer rates and indicate that sulfate is reoxidized below the
420 sediment surface by reaction with reactive iron (Thang et al., 2013). Whole-core methane fluxes ranged
421 from $-0.1 \pm 0.05 \text{ mmol m}^{-2} \text{ d}^{-1}$ (February) to $-1.2 \pm 0.6 \text{ mmol m}^{-2} \text{ d}^{-1}$ (August) at station B1 and from -
422 $0.3 \pm 0.1 \text{ mmol m}^{-2} \text{ d}^{-1}$ (April 2012) to $-19.9 \pm 7.8 \text{ mmol m}^{-2} \text{ d}^{-1}$ (August) at station H6 (Figure 5, Table
423 2). However, the following year, a significantly higher methane flux of $3.9 \text{ mmol m}^{-2} \text{ d}^{-1}$ was measured
424 in April 2013 at station H6. Significant upward diffusive methane fluxes ranged from $0.02 \text{ mmol m}^{-2} \text{ d}^{-1}$
425 d^{-1} (February 2012) to $0.3 \text{ mmol m}^{-2} \text{ d}^{-1}$ (August) at Station B1 and from 0.5 (February) to 2.3 mmol m^{-2}
426 d^{-1} (August) at station H6. Thus, there was a generally poor agreement between whole-core and
427 diffusive flux-derived methane fluxes. The large discrepancy between the August 2012 diffusive flux
428 and whole-core flux is best explained that the cores were taken from sediments with different organic
429 carbon contents. Since several Multicorer casts were taken per station and the vessel's positioning
430 ability in strong winds was at best tens of meters, sediment heterogeneity can possibly explain this
431 difference. The very high whole-core flux value measured in August 2012 at Station H6 is likely due to
432 ebullition during the incubation at ambient air pressure and oversaturation of the porewater with respect
433 to atmospheric pressure.

434

435 **4. Discussion**

436 **4.1 Bottom water temperature and salinity**

437 Correlations between biogeochemical rates and fluxes with bottom water temperatures in
438 Himmerfjärden between April 2012 and February 2013 were weak for the period April-October, and
439 forced by the low rates in the coldest observation period in early February 2013. All R values
440 calculated for pairs of temperature versus rate/flux were less than 0.2 and not consistent for the fluxes
441 of oxygen, methane, and sulfate indicating that additional environmental controlling factors played a
442 role. It is likely that the microbial community involved in the cycling of methane and sulfur species in
443 Himmerfjärden sediment is temperature-sensitive, and that the low rates in February 2013 are due to
444 the 3°C temperature drop in bottom water from October 2012 to February 2013 (Table 1). This would
445 be consistent with rate observations in comparable environments by Treude et al (2005a), Abril and
446 Iversen (2002), Crill and Martens (1983), and Westrich and Berner (1988), and is also supported by
447 studies of the microbial community composition of estuarine sediments that showed variations as a
448 function of temperature (e.g., Zhang et al 2014). Regulation of methane fluxes largely by temperature
449 implies that methane oxidation in Himmerfjärden sediment is less temperature-sensitive than
450 methanogenesis preventing methane-oxidizing bacteria from keeping up with the enhanced methane
451 flux during summer. This requires significantly higher temperature stimulation of methanogenesis than
452 methane oxidation, the lack of an electron acceptor, or successful competition for the same electron
453 acceptor by other organisms than methane-oxidizing bacteria. Publications from lake environments and
454 terrestrial environments suggest that aerobic methane-oxidizing bacteria may indeed be less
455 temperature-sensitive than methanogens (King, 1992; Wik et al., 2014; Nguyen et al., 2011). However,
456 this argument is not directly applicable to marine habitats. In case of anaerobic methane oxidation, it is
457 difficult to argue for a physiological temperature disadvantage of methane oxidizers compared to
458 methanogens, because of the tight coupling between sulfate reduction and methane oxidation, the
459 phylogenetic proximity of ANME to known methanogenic Archaea (Knittel and Boetius, 2009), and

460 similarities in membrane composition of ANME and methanogenic Archaea (Wegener et al., 2012).
461 However, temperature control may not manifest itself by direct kinetic or bioenergetic regulation, but
462 indirectly through the influence on competing processes, e.g., sulfate reduction and methanogenesis.
463 Further, microbial community composition and biogeochemical rates often cannot be directly
464 established from binary relationships with temperature, since other physical and chemical parameters
465 such as salinity, bottom water oxygen concentrations, organic carbon accumulation also vary
466 seasonally. Of these, salinity is not considered to be important for the present study, because the annual
467 range in Himmerfjärden bottom water was only between 5.4 and 7 ‰, which is too small to affect the
468 major electron acceptor and carbon degradation pathways.

469

470 **4.2 Effects of organic matter composition and sedimentation**

471 Organic carbon concentrations in Himmerfjärden are similar to other fjord- and fjärd-type estuarine
472 sediments (Bianchi, 2007; Smith et al., 2015). Primary organic carbon export in Himmerfjärden varies
473 strongly on both seasonal and interannual timescales (Blomqvist and Larsson, 1994). The major export
474 periods occur during the spring phytoplankton bloom after ice breakup from March-April until early
475 May, during a late-summer cyanobacterial bloom in August, and after a weaker, secondary
476 phytoplankton bloom in September (Bianchi et al., 2002; Zakrisson et al., 2014; Harvey et al., 2015).
477 Terrestrial-derived organic carbon that is not derived from the sewage treatment plant plays only a
478 minor role in this system, because no major rivers enter the system and surface rainwater runoff is low.
479 Based on sediment trap studies, the annual organic carbon flux in Himmerfjärden varies by more than
480 an order of magnitude at station B1 and by about a factor of 3 in the inner parts of Himmerfjärden
481 (Blomqvist and Larsson, 1994). Observations over a 5-year period by Blomqvist and Larsson (1994)
482 indicated that primary organic carbon dominates organic sedimentation in the spring and summer at
483 station B1, whereas station H6 is characterized by a spring term dominance of primary carbon
484 deposition, but a much greater contribution of resuspended organic material to organic sedimentation
485 during the fall (Blomqvist and Larsson, 1994).

486 A second effect to be considered is that stations B1 and H6 are located in bathymetric depressions. H6
487 is in the center of a sub-basin separated from the outer Himmerfjärd by a sill (Fig. 1). Likewise, Station
488 B1 is located in a small depression at the head of a submarine channel that opens to the Baltic Sea.
489 Fine-grained and reworked organic-rich material preferentially accumulates in these depressions
490 (Jonsson et al., 2003). Because of the importance of resuspended organic material for the vertical mass
491 flux and bioturbation, the annual variability in the organic matter composition at the sediment surface
492 varies year-round only between 5 and 6 % OC with relatively constant C/N ratios between 7.9 and 9.1
493 at Station B1 and 8.3 and 9.2 at Station H6 (Bonaglia et al., 2014). Organic mass accumulation rates in
494 the accumulation bottoms based on ^{210}Pb dating are reported between 3.3 and 9.5 mol m⁻² y⁻¹ (Thang et
495 al., 2013; Karlsson et al., 2010). The combined effect of these sedimentation characteristics is that
496 temporal variability in the settling primary organic carbon flux above the sediment surface is low,
497 which reduces the overall temporal variability in organic carbon amount and composition and thereby
498 in carbon mineralization rates. This small temporal variability is further influenced by macrofauna
499 bioturbation in the top 2-3 cm of sediment in this area, foremost by the bivalve *Macoma baltica*, the
500 arthropod *Pontoporeia femorata*, and the polychaete *Marenzelleria* (Bonaglia et al., 2014). Although
501 macrofauna is largely absent at Station H6, sediment is also mixed at station H6 by bioturbating
502 meiofauna (mostly ostracods) (Bonaglia et al., 2014).

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

510

511 **4.3 Methane fluxes, sulfate reduction and methane oxidation**

512 Preferential accumulation of sediment in the bathymetric depressions of the inner Himmerfjärden
513 results in very high sedimentation rates between 0.9 and 1.3 cm/yr (Thang et al., 2013; Bianchi et al.,
514 2002). In such sediments organic carbon burial and transfer of organic matter into the methanogenic
515 zone is efficient and will occur within 20 to 30 years. As a consequence of the low bottom water
516 salinity of 6 ‰ of the Baltic Sea at this latitude, seawater sulfate concentrations are less than 7 mM
517 and, by comparison with normal seawater, a comparatively lesser amount of organic matter can be
518 degraded by bacterial sulfate reduction (Thang et al., 2013). Consequently, compared to normal marine
519 sediments a larger proportion of organic matter undergoes anaerobic microbial degradation terminating
520 in methanogenesis, which generates a high upward flux of methane into the sulfate-containing zone.
521 Organoclastic sulfate-reducing bacteria will compete for the available sulfate with sulfate-reducing
522 bacteria involved in the anaerobic oxidation of methane (Dale et al., 2006; Jørgensen and Parkes,
523 2010). Thermodynamic and kinetic constraints decide on the outcome between these two competing
524 processes. Dale et al. (2006) suggested that due to lower winter temperatures and greater sulfate
525 availability in the sulfate-methane transition zone in winter, the thermodynamic driving force for
526 anaerobic methane oxidation increases allowing for a greater proportion of anaerobic methane
527 oxidation coupled to sulfate reduction in the winter. In the summer and fall, higher temperatures and
528 sulfate limitation may favor organoclastic sulfate reduction and methanogenesis while limiting the
529 anaerobic oxidation of methane. Most importantly, however, their analysis showed that due to
530 thermodynamic constraints and slow growth rates of the methane-oxidizing archaea the microbial
531 biomass does not change significantly over a year. These conceptual modelling results can be tested
532 with our Himmerfjärden data.

533 Sulfate reduction rates, particularly at H6, demonstrate how strongly bottom-water oxygen controls
534 organic matter mineralization. In the spring, summer, and fall sulfate reduction was at its maximum in
535 the first two centimeters of the sediments (Fig. 3 e, f, g). In February, reduced organic carbon input and

536 higher oxygen concentrations resulted in lower sulfate reduction rates and a downward displacement of
537 the maximum rates sediment confining methane production to greater depths in the sediment.

538 The decrease in oxygen uptake matches well with the decrease in methane fluxes at the two stations in
539 winter, which suggests an impact of oxygen on methane cycling (Table 2, Figure 5). Higher oxygen
540 levels enhance bioturbation and oxygen uptake by the abundant macro- and meiofauna (Norkko et al.,
541 2015), but the mixing of sediment also affects methane transport to the water column, as the main
542 transport process shifts from diffusion to advection. This effect is likely the main cause for the winter
543 decrease in methane fluxes and concentrations. More aerated conditions indirectly enhance methane
544 removal by sustaining aerobic methanotrophs (Valentine 2011). It is plausible that, as in other brackish
545 coastal sediments, aerobic methanotrophs at the surface of Himmerfjärden sediments consume a
546 significant part of upward-diffusing methane that was not oxidized by anaerobic methane oxidation
547 (McDonald et al 2005, Moussard et al 2009, Treude et al 2005a).

548 Published benthic methane fluxes for estuaries with similar salinities have a reported range of 0.002 to
549 $0.25 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Abril and Iversen, 2002; Martens and Klump, 1980; Sansone et al., 1998; Zhang et
550 al., 2008; Borges and April, 2012; Martens et al., 1998). The methane fluxes derived from our core
551 incubations ($0.1\text{-}3.9 \text{ mmol m}^{-2} \text{ d}^{-1}$, ignoring the potentially biased value of $19.9 \text{ mmol m}^{-2} \text{ d}^{-1}$) and the
552 corresponding diffusive fluxes ($0.01\text{-}2.4 \text{ mmol m}^{-2} \text{ d}^{-1}$) were high compared to these published fluxes.

553 However, our fluxes are consistent with fluxes based on porewater gradients by Thang et al. (2013) that
554 were between 0.3 and $1.1 \text{ mmol m}^{-2} \text{ d}^{-1}$ at 3 nearby stations measured in May 2009.

555 A conspicuous property of all porewater profiles at station H6, with the exception of the February 2013
556 sampling period, was the absence of a curvature in most methane concentration profiles, which would
557 be expected for net methane oxidation by aerobic and anaerobic methane oxidation (Martens et al.,
558 1998). Most concentration profiles of sulfate and methane at Station H6 overlapped without a
559 significant change in the methane concentration gradient. A similar observation has been made earlier
560 for other Himmerfjärden sediments (Thang et al., 2013), and has also been reported for sediments of
561 the northwestern Black Sea shelf (Knab et al., 2009) and in organic-rich shelf sediment of the

562 Namibian upwelling system (Brüchert et al., 2009). Inefficient methane oxidation is also evident from
563 the diffusive fluxes, which showed that the upward fluxes of methane into the sulfate-methane
564 transition zone were only marginally higher than the methane fluxes to the sediment surface indicating
565 little attenuation of the methane flux in the sulfate-methane transition zone (Table 2). One possible
566 explanation for this phenomenon is therefore that rates of sulfate reduction–coupled anaerobic methane
567 oxidation, except for the winter months, were low compared to the organoclastic sulfate reduction rate.
568 An alternative explanation of our observations could be that the methane concentration gradients were
569 affected by the presence of rising methane bubbles (Haeckel et al., 2007), or that bioturbation and
570 bioirrigation linearized the concentration profiles (Dale et al., 2013). However, we do not favor these
571 latter interpretations because of the absence of large macrofauna at station H6, the fact that methane
572 concentrations were below the in-situ saturation concentration of methane, and the fast porewater
573 methane sampling method preventing significant gas formation.

574 An analysis of the cumulative distribution of ^{35}S -SRR with depth at station H6 provides clues to the
575 proportion of organoclastic relative to anaerobic methane oxidation-coupled sulfate reduction at Station
576 H6 (Figure 6 e-h). In contrast to station B1, where an exponentially decreasing portion of sulfate
577 reduction contributed to the total sulfate reduction at depth, at station H6 a distinct steepening in the
578 cumulative sulfate reduction is observed below 10 cm in April, August, and October. As discussed
579 above, we do not attribute the steepening observed in February 2013 to the same process, because
580 sulfate was still present in abundance at this depth and methane concentrations were low and without
581 any apparent change in gradient in this depth zone. The gradient in organoclastic sulfate reduction can
582 be described by an exponential function (Jørgensen and Parkes, 2010),

$$583 \quad {}^{35}\text{SRR} = y z^{-b} \quad (6)$$

584 where z is depth (cm) and y and b are regression coefficients (Jørgensen and Parkes, 2010). Fitting the
585 sulfate reduction rates investigated here to such a function yielded exponential coefficients b between
586 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
587 was found for February 2013, when sulfate penetrated the deepest into the sediment (Table 4). Since

588 the upward flux of methane provides an additional energy source to sulfate-reducing bacteria, total
589 sulfate reduction rates are expected to increase in the sulfate-methane transition zone. If substantial
590 AOM-coupled and organoclastic sulfate reduction occur at the same depths the total ^{35}S -sulfate
591 reduction rate depth gradient will be lower and the exponential coefficient b will be smaller than for a
592 setting without significant AOM. The difference between the exponential coefficients for the different
593 observation times can be used to calculate the variation in the contribution of AOM to the total sulfate
594 reduction rate. At station H6, between 5 % (August 2012) and 20% (April 2012) of the total sulfate
595 reduction can be associated with anaerobic methane oxidation. A comparison of the above method with
596 the ^{35}S -sulfate reduction rates integrated over the length of the H6 sediment cores with the rates
597 integrated in the AOM zone also indicated that >20% of sulfate reduction at H6 was supported by
598 anaerobic methane oxidation (Table 2). In near-shore continental margin sediments worldwide, the
599 fraction of methane-driven sulfate reduction varies between locations and accounts for 3-40% of total
600 sulfate reduction, with 10% possibly representing a global mean value (Jørgensen and Kasten, 2006).
601 The average 20% contribution calculated here falls in the upper range of these values and is similar to
602 values reported before for one of the monitoring stations within Himmerfjärden (Thang et al., 2013)
603 and also for a very productive Chilean slope sediment (8-24 %) (Treude et al 2005b). The good match
604 between the upward fluxes of methane in the sulfate-methane transition zone and the measured sulfate
605 reduction rates in the transition zone also indicate that other proposed electron acceptors for anaerobic
606 methane oxidation such as iron are unimportant in these sediments (Beal et al., 2009; Egger et al.
607 2014).

608

609 **4.4 Temporal variability in hydrostatic pressure**

610 The abrupt decrease in porewater methane concentrations from October 2012 to early February 2013
611 and the subsequent increase in April 2013 cannot be explained by variation in methane oxidation alone,
612 because the temporal change in porewater methane concentration was large compared to the inferred
613 methane oxidation rates based on fluxes in and out of the AOM zone. In addition, except for

614 downward-diffusing sulfate, there was no significant other electron acceptor present at depth. It is
615 unlikely that rates of methanogenesis would have decreased significantly between the fall and the
616 winter and resumed again in the spring because of the sedimentological characteristics described above
617 and the small difference in sediment temperatures for February and April (Table 1). Changes in organic
618 matter sedimentation at the sediment surface also have no significant influence on methanogenesis rates
619 in buried sediment and cannot explain the sudden decrease in methane concentration at depth. An
620 alternative explanation for the changes in methane concentrations is required. A possible explanation
621 could be that changes in upward transport of methane are due to variability in hydrostatic pressure and
622 the associated diffusive and advective upward transport of methane from depth. The free gas depth of
623 methane is thought to follow changes in hydrostatic pressure and temperature (Mogollon et al., 2011;
624 Toth et al., 2015). An estimated 10% of the fine-grained sediments in the Stockholm archipelago area
625 are underlain by pockets of free methane (Persson and Jonsson, 2000) and these free gas pockets are
626 preferentially located in areas with the thickest postglacial mud accumulation, generally in the center of
627 the sub-basins and along fault lineaments (Söderberg and Floden, 1992). Based on sub-bottom
628 echosounder profiling, the surface of the free gas zone in accumulation areas in Himmerfjärden and
629 other areas of the Stockholm archipelago is between 1 and 3 meter depth (Söderberg and Floden,
630 1991). During low sealevel stand the free gas zone is expected to migrate closer to the sediment
631 surface, whereas during high sealevel the free gas zone is depressed into the sediment. The total
632 variation in sealevel is related to air pressure, prevailing wind directions, precipitation, and the balance
633 of saltwater entry through the Danish straits and freshwater discharge from rivers entering the Baltic
634 Sea (Andersson, 2002). Additional effects are caused by local coastal bathymetry, current flow, and,
635 possibly, and local submarine groundwater discharge. These multiple parameters result in complex
636 subsurface hydrology and may produce sealevel fluctuations that can be as much as 50 cm, sufficient to
637 explain the changes in methane concentrations observed here. Unfortunately, local data within
638 Himmerfjärden on sealevel fluctuations are not available for our respective sampling locations, and
639 regional sealevel stands should not be directly applied to the sample sites.

640 The above discussion demonstrates that a variety of processes interact in these fjord sediments to
641 produce the observed methane fluxes. It is beyond the scope of this paper to develop a unifying model
642 against which the variability of the observed fluxes can be tested, but we would like to point out that
643 the local coastal hydrography and hydrogeology would need to be accounted for in such a coupled
644 physical biogeochemical model. To our knowledge, sufficient subsurface geophysical data are currently
645 not available to establish appropriate physical boundary conditions for such a model. Detailed
646 geophysical analysis of the subsurface structure at high vertical resolution together with long-term
647 monitoring of the porewater chemistry would shed new light on the coupling between subsurface
648 hydrology and methane emissions.

649

650

651 **5 Conclusions**

652 A greater understanding of methane emissions from estuarine and coastal sediments is important to
653 estimate the contribution of these environments to global marine methane fluxes. High benthic fluxes
654 of methane from these sediments showed that total methane oxidation was relatively inefficient, despite
655 the fact that anaerobic methane oxidation contributed up to 20% to total sulfate reduction. Of the
656 different environmental regulators, bottom water oxygen had the strongest influence for the regulation
657 of methane emissions. Oxygen availability directly enhanced aerobic organic matter mineralization by
658 shifting the redox cascade in the sediments and indirectly by stimulating meiofauna and macrofauna
659 activity thereby stimulating both the aerobic carbon mineralization and oxidative recycling of sulfate.
660 The annual variability in sediment methane concentrations and benthic methane fluxes indicate that the
661 annual environmental changes at these near-shore, but relatively deep-water localities are considerable.
662 Very few data on sediment biogeochemical processes are currently available for aerobic and anaerobic
663 carbon mineralization and methane cycling during winter months when ice cover inhibits access and

664 sampling. Process rates inferred from sampling during open-water conditions over the whole year are
665 therefore likely overestimates.

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

670

671

672 **6. Author contribution**

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

676

677 **7. Data availability**

678 The data are available from the second author upon request.

679

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687

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- 847

848 **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 CH₄ and SO₄²⁻ fluxes, depth-integrated ³⁵SRR, and total oxygen uptake (TOU).

Station	Sampling time	Flux (mmol m ⁻² d ⁻¹)						Integrated ³⁵ S-SRR (n=3)
		TOU whole core incubation (n=4)	CH ₄ whole core incubation (n=4)	CH ₄ Diffusive flux to sediment surface (n=1)	CH ₄ Diffusive flux into SMTZ (n=1) ²	SO ₄ ²⁻ Diffusive flux into sediment (n=1)	³⁵ S-SRR integrated over AOM ³ zone (n=3)	
B1	April 2012	19.7±2.5	-0.10±0.05	-0.1		0.4	no AOM zone ⁴	2.3±0.6
	August 2012	22.5±2.9	-1.2±0.6	-(0.01)		0.8	no AOM zone ⁴	0.5±0.1
	October 2012	21.1±2.7	No data	-0.3		1.4	no AOM zone ⁴	2.0±0.0.5
	February 2013	12.0±1.5	-0.1±0.05	-0.02		0.2	no AOM zone ⁴	2.2±0.6
H6	April 2012	33.5±3.5	-0.3±0.1	-1.7	-2.8	2.6	(10-18 cm) = -2.8±0.7	11.6±2.9
	April 2013		-3.9 ±0.7 ¹					
	August 2012	26.9±2.8	-19.9±7.8 ⁵	-2.3	-2.6	2.7	(10-18 cm) = -2.8±0.7	11.7±2.9
	October 2012	25.9±2.7	-1.0	-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	-0.5	-0.4	1.3	no AOM zone ³	9.2±2.3

858 ¹ whole core incubation was performed in April 2013; Diffusive fluxes were calculated for samples collected in April 2012;

859 ² SMTZ - sulfate methane transition zone, ³ AOM zone – zone of anaerobic oxidation of methane, ⁴ no AOM zone means

860 that AOM zone was probably deeper than the core length; ⁵ potentially elevated due to depressurization/ex-solution

861 effect during core incubation at atmospheric pressure;

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

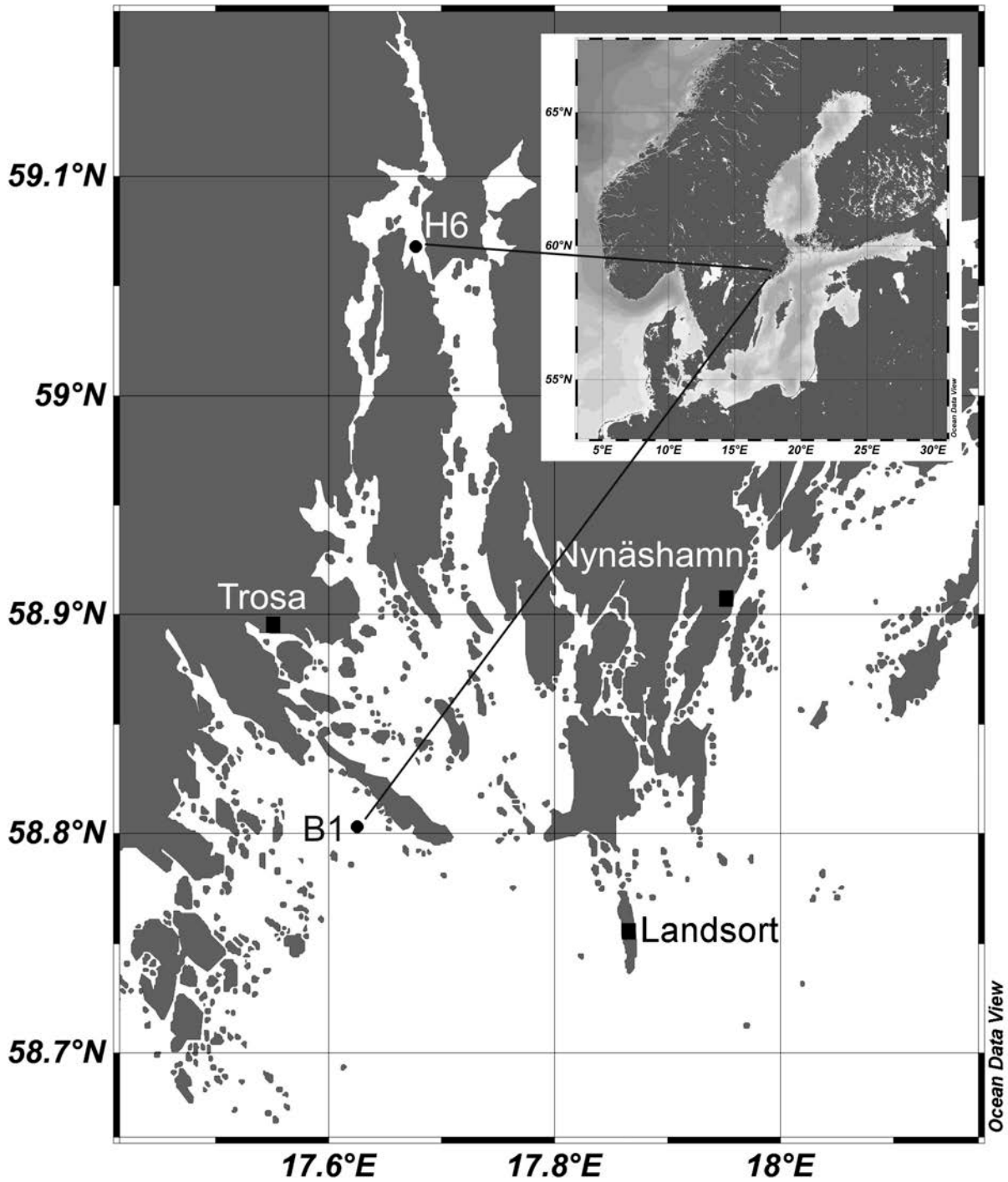
Station	Sampling time	Exponential coefficient (a)	Exponential coefficient (b)
B1	April 2012	147.0	-1.4
	August 2012	11.7	-0.9
	October 2012	16.0	-0.4
	February 2013	33.5	-0.8
H6	April 2012	18.6	-0.5
	August 2012	37.4	-0.5
	October 2012	133.2	-0.8
	February 2013	25.0	-0.4

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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).

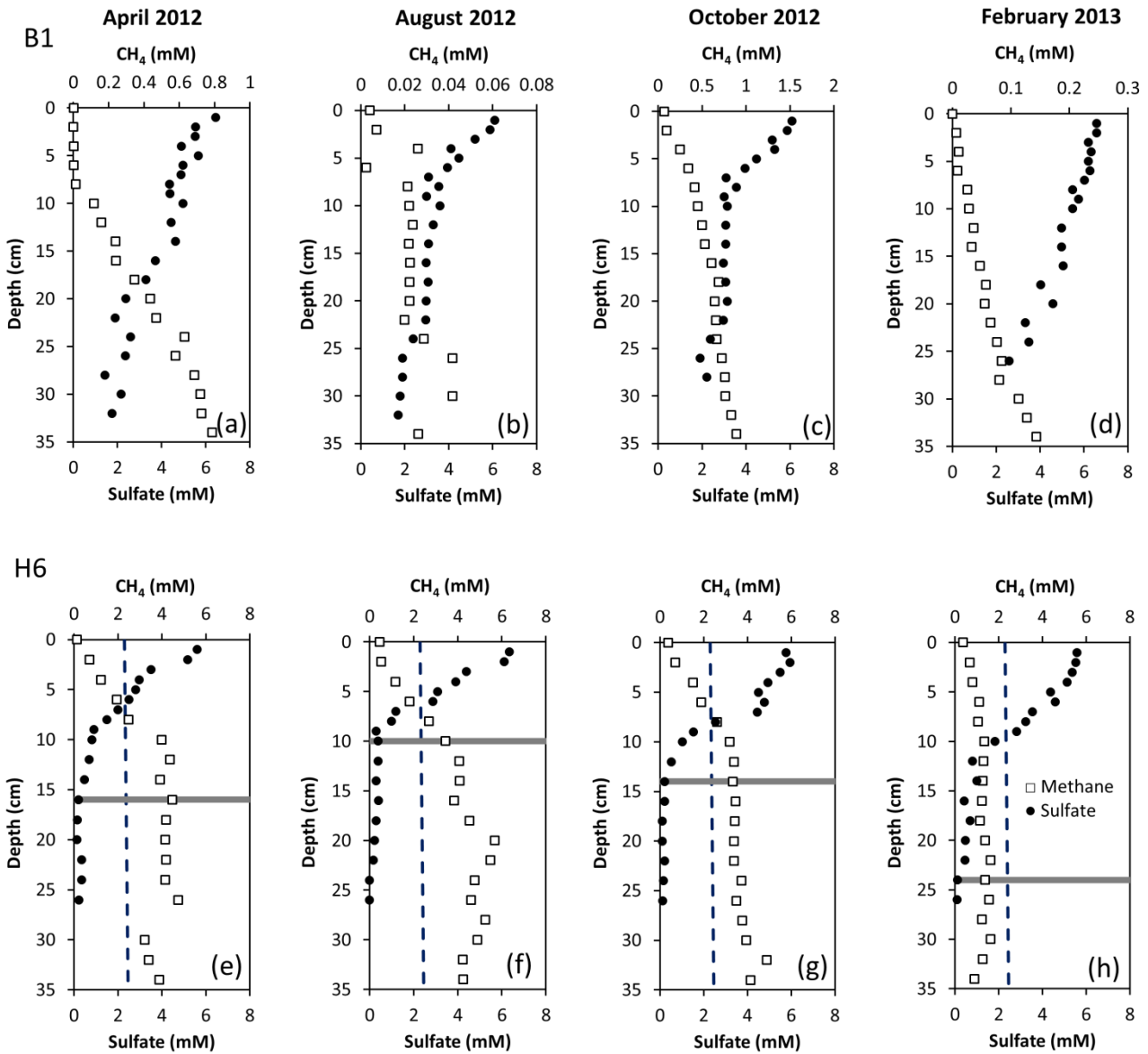


Figure 2. Porewater profiles of total methane and sulfate at Station B1 (a-d) and Station H6 (e-h) for the different sampling periods. The grey line marks the initial minimum sulfate concentration depth. Dashed lines indicate the methane saturation concentration at 1 atm pressure (grey) at the time of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

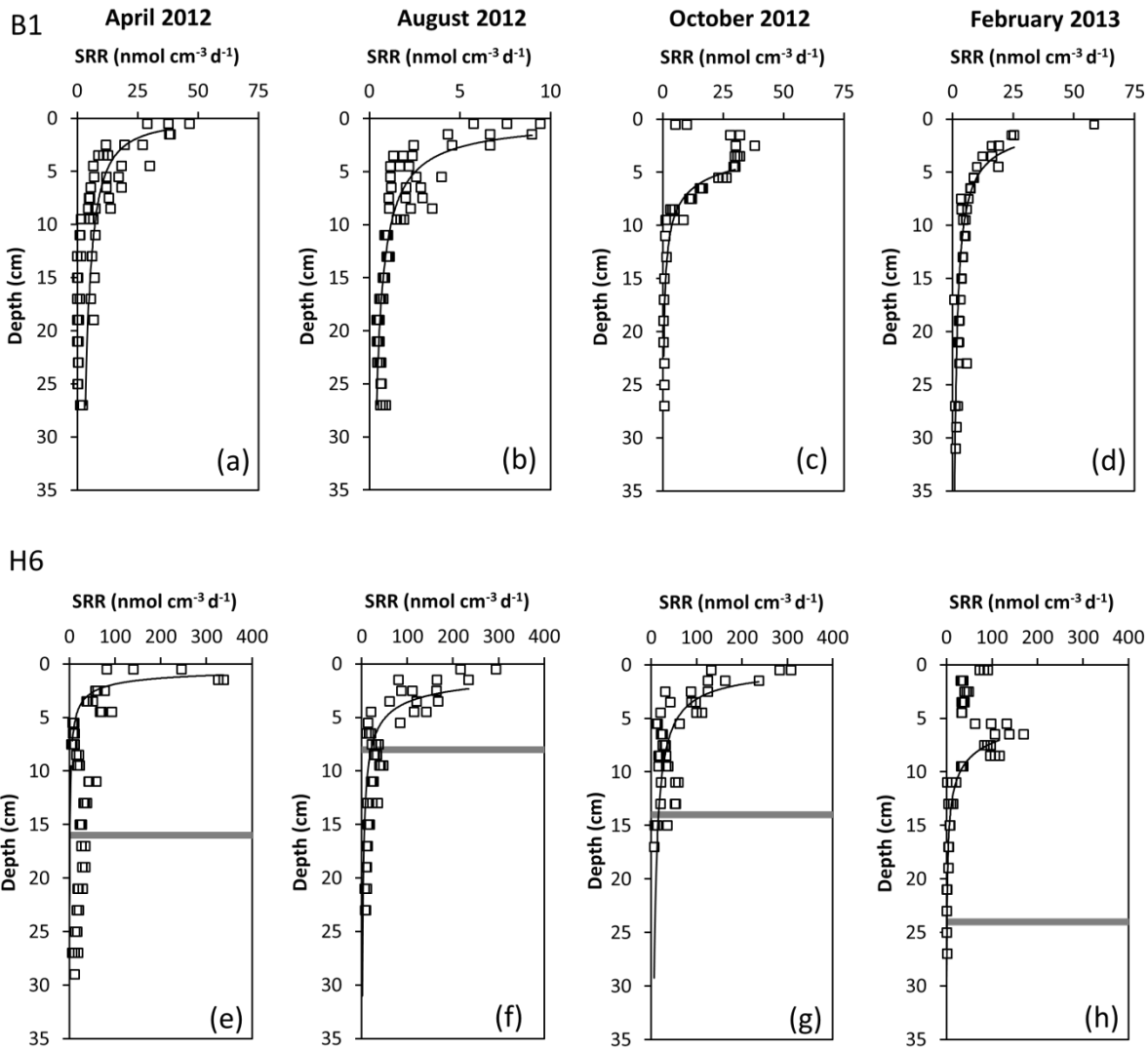


Figure 3. Depth gradients of bacterial sulfate reduction rates (SRR) measured with ³⁵S-sulfate at Station B1 (a-d) and Station H6 (e-h) for the different sampling periods. Black lines show the regression results to a power function of the form $y = ax^{-b}$. The grey line marks the initial minimum sulfate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

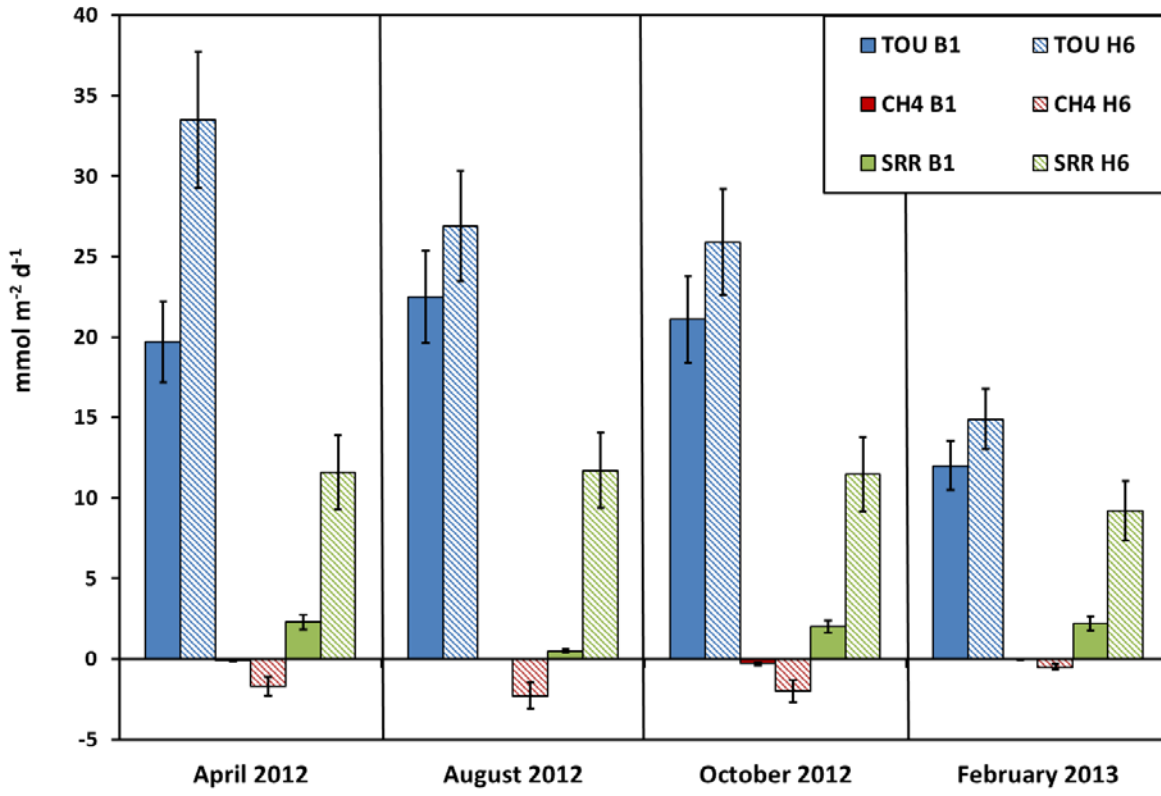


Figure 4. Comparison of benthic fluxes ($\text{mmol m}^{-2} \text{d}^{-1}$) for sulfate (SO_4), methane (CH_4), and oxygen (TOU) for the different sampling periods.

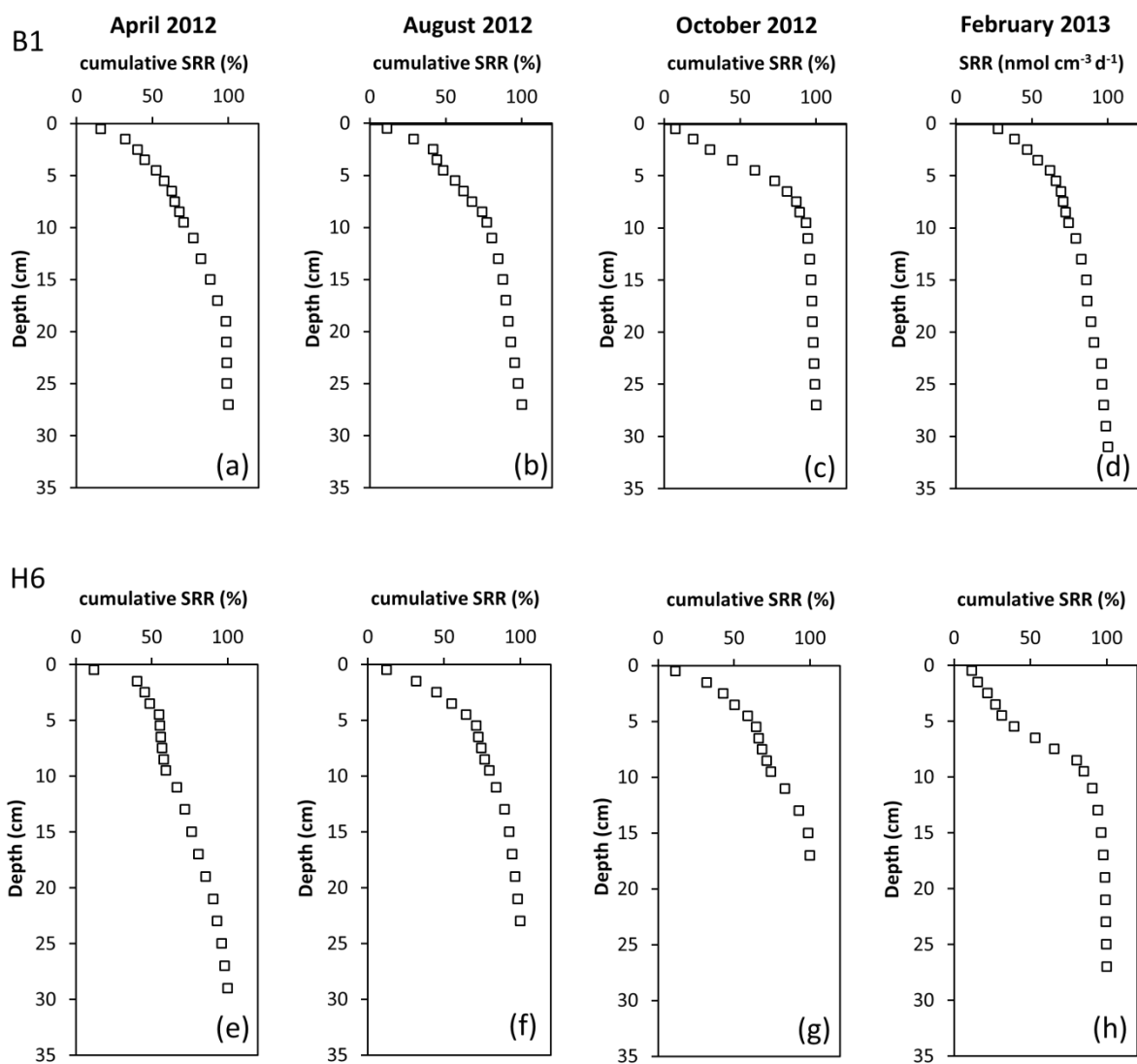


Figure 5. Depth distribution of sulfate reduction rate expressed as cumulative percentage at Station B1 (a-d) and Station H6 (e-h) for the different sampling periods. The grey line marks the initial minimum sulfate concentration depth.