



# Microbial methanogenesis in the sulfate-reducing zone of surface sediments traversing the Peruvian margin

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**Abstract.** We studied the concurrence of methanogenesis and sulfate reduction in surface sediments (0–25 cm below sea floor) at six stations (70, 145, 253, 407, 990 and 1024 m) along the Peruvian margin (12° S). This oceanographic region is characterized by high carbon export to the seafloor creating an extensive oxygen minimum zone (OMZ) on the shelf, both factors that could favor surface methanogenesis. Sediments sampled along the depth transect traversed areas of anoxic and oxic conditions in the bottom-near water. Net methane production (batch incubations) and sulfate reduction (<sup>35</sup>S-sulfate radiotracer incubation) were determined in the upper 0–25 cm b.s.f. of multiple cores from all stations, while deep hydrogenotrophic methanogenesis (> 30 cm b.s.f., <sup>14</sup>C-bicarbonate radiotracer incubation) was determined in two gravity cores at selected sites (78 and 407 m). Furthermore, stimulation (methanol addition) and inhibition (molybdate addition) experiments were carried out to investigate the relationship between sulfate reduction and methanogenesis.

Highest rates of methanogenesis and sulfate reduction in the surface sediments, integrated over 0–25 cm b.s.f., were observed on the shelf (70–253 m, 0.06–0.1 and 0.5–4.7 mmol m<sup>-2</sup> d<sup>-1</sup>, respectively), while lowest rates were discovered at the deepest site (1024 m, 0.03 and 0.2 mmol m<sup>-2</sup> d<sup>-1</sup>, respectively). The addition of methanol resulted in significantly higher surface methanogenesis activity, suggesting that the process was mostly based on non-competitive substrates – i.e., substrates not used by sulfate reducers. In the deeper sediment horizons, where competition was probably relieved due to the decrease of sulfate, the usage of competitive substrates was confirmed by the detec-

tion of hydrogenotrophic activity in the sulfate-depleted zone at the shallow shelf station (70 m).

Surface methanogenesis appeared to be correlated to the availability of labile organic matter (C/N ratio) and organic carbon degradation (DIC production), both of which support the supply of methanogenic substrates. A negative correlation between methanogenesis rates and dissolved oxygen in the bottom-near water was not obvious; however, anoxic conditions within the OMZ might be advantageous for methanogenic organisms at the sediment-water interface.

Our results revealed a high relevance of surface methanogenesis on the shelf, where the ratio between surface to deep (below sulfate penetration) methanogenic activity ranged between 0.13 and 10<sup>5</sup>. In addition, methane concentration profiles indicated a partial release of surface methane into the water column as well as consumption of methane by anaerobic methane oxidation (AOM) in the surface sediment. The present study suggests that surface methanogenesis might play a greater role in benthic methane budgeting than previously thought, especially for fueling AOM above the sulfate–methane transition zone.

## 1 Introduction

Microbial methanogenesis represents the terminal step of organic matter degradation in marine sediments (Jørgensen, 2006). The process is entirely restricted to a small group of prokaryotes within the domain of the Archaea (Thauer, 1998). Methanogens produce methane from a narrow spec-

trum of substrates, primarily carbon dioxide (CO<sub>2</sub>) and hydrogen (H<sub>2</sub>) (hydrogenotrophic pathway), as well as acetate (acetoclastic pathway) (Zinder, 1993). In addition, methanol or methylated compounds such as methylamine can be utilized (methylotrophic pathway) (Oremland and Polcin, 1982; Buckley et al., 2008; Zinder, 1993; King et al., 1983). Substrates for methanogenesis are produced during depolymerization and fermentation of organic macromolecules (e.g., sugars, vitamins, amino acids) to smaller monomeric products (Jørgensen, 2006; Schink and Zeikus, 1982; Neill et al., 1978; Donnelly and Dagle, 1980).

Acetoclastic and hydrogenotrophic methanogenesis are predominantly found in deeper sediment zones below sulfate penetration, owing to the more effective utilization of H<sub>2</sub> and acetate by sulfate reducers due to their higher substrate affinity (Oremland and Polcin, 1982; Jørgensen, 2006). Methanogens can avoid competition with sulfate reducers by the utilization of non-competitive substrates, such as methanol or methylamines (Oremland and Polcin, 1982; King et al., 1983). Facilitated by the usage of such non-competitive substrates, sulfate reduction and methanogenesis were found to co-occur in sulfate-containing salt marsh sediments (Oremland et al., 1982; Buckley et al., 2008; Senior et al., 1982). Concurrent activity of sulfate reduction and methanogenesis in the marine environment has mostly been postulated for organic-rich sediments (Mitterer, 2010; Jørgensen and Parkes, 2010; Treude et al., 2009, 2005a; Hines and Buck, 1982; Crill and Martens, 1986); however, details on the magnitude and environmental controls of surface methanogenesis are still poorly understood (Holmer and Kristensen, 1994; Ferdelman et al., 1997).

In a study from Eckernförde Bay, southwestern Baltic Sea, considerable *in vitro* methanogenic activity was observed in samples taken from 5 to 40 cm sediment depth (Treude et al., 2005a). Although *in vitro* activity was measured in sulfate-free setups, methanogenic activity coincided with zones of *in situ* sulfate reduction. The authors concluded a coexistence of the two types of organisms, which could be enabled through either the usage of non-competitive substrates, dormancy of methanogens until phases of sulfate depletion, and/or temporal or spatial heterogeneity in the sediments. Eckernförde Bay sediments feature a high input of organic matter due to a shallow water depth (~30 m) and pronounced phytoplankton blooms in spring, summer and fall (Smetacek, 1985). Furthermore, seasonal hypoxia (O<sub>2</sub> < 90 μM) or even anoxia (O<sub>2</sub> = 0 μM) occur in the deep layers of the water column caused by stratification and degradation of organic matter (Bange et al., 2011). Oxygen-depleted conditions in the bottom water together with frequent input of fresh organic matter possibly favors methanogenesis in surface sediment by offering reduced conditions and non-competitive substrates. As non-competitive substrates can be derived from organic osmolytes such as betaine or dimethylsulfoniopropionate (DMSP), a high load of organic matter (e.g., through sedimentation of phytoplankton blooms) can increase the

availability of non-competitive substrates (Zinder, 1993; Van Der Maarel and Hansen, 1997). Similarly, methanogenesis activity was observed within the sulfate-reducing zone of organic-rich sediments from the seasonally hypoxic Limfjorden sound, northern Denmark (Jørgensen and Parkes, 2010; Jørgensen, 1977).

The environmental relevance of surface methanogenesis is hitherto unknown. Its closeness to the sediment–water interface makes it a potential source for methane emissions into the water column, unless the methane is microbially consumed before escaping the sediment (Knittel and Boetius, 2009). Methane escapes the sediment either by diffusion or, when methane saturation is exceeded, in the form of gas bubbles (Whiticar, 1978; Wever and Fiedler, 1995; Judd et al., 1997; Dimitrov, 2002). The fraction of methane released to the water column that reaches the atmosphere mainly depends on water depth, as methane is also consumed within the water column through aerobic microbial oxidation (Reeburgh, 2007; Valentine et al., 2001). Thus, shallow coastal areas have higher methane emission potentials than the open ocean (Bange et al., 1994) and a greater potential to contribute to methane-dependent atmospheric warming (IPCC, 2014).

In the present study we focused on the upwelling region off the Peruvian coast, which is another example of an environment where both factors that potentially favor surface methanogenesis convene – i.e., a high export of organic carbon and low dissolved oxygen concentrations in the bottom water. This upwelling region represents one of the most productive systems in the world oceans, creating one of the most intense oxygen minimum zones (OMZs, Kamykowski and Zentara, 1990; Pennington et al., 2006). Oxygen concentrations in waters impinging on the seafloor are below 20 μM or even reach anoxia. Research on surface sediment methanogenesis in upwelling regions is scarce and its potential role in the carbon cycling of the Peruvian OMZ is completely unknown. In a study from the central Chilean upwelling area (87 m water depth, 0.5–6 cm sediment depth), low methane production rates were detected despite high sulfate reduction activity, when the non-competitive substrate trimethylamine was offered (Ferdelman et al., 1997). The authors concluded that the prevailing methanogens were competing with sulfate reducers for H<sub>2</sub> and with acetogens for methylamines, explaining the overall low methanogenesis activity observed (Ferdelman et al., 1997).

Even though the Chilean and Peruvian OMZs are connected, commonly known as OMZ in the eastern South Pacific Ocean (ESP) (Fuenzalida et al., 2009), the core of the ESP-OMZ is centered off Peru with an upper boundary at < 100 m and a vertical distribution to > 600 m versus a thinner OMZ band off Chile constrained between 100 and 400 m water depth (Fuenzalida et al., 2009). Anoxic conditions in the water column of the OMZ core (and therewith a lack of bioirrigating macrofauna introducing oxygen into the sediments, Kristensen, 2000) together with the high export rates of la-

bile organic carbon to the seafloor (Reimers and Suess, 1983; Dale et al., 2015) provide favorable conditions for methanogenesis activity in surface sediments, thus increasing the potential for benthic methane emissions.

Here, we provide first insights into surface methanogenesis in sediment cores (< 30 cm b.s.f. = centimeters below seafloor) taken along the Peruvian shelf and margin. We hypothesize that methanogenesis coexists with sulfate reduction through the utilization of non-competitive substrates. In addition, we postulate that surface methanogenesis depends on the quantity and quality (= freshness) of organic carbon, and the concentrations of dissolved oxygen in the bottom water. We therefore expect spatial variability of surface methanogenesis along the continental shelf and margin. The observed methanogenic activity will be compared to methane concentrations in the bottom-near water to discuss the potential relevance of surface methanogenesis for methane emissions into the pelagic zone.

## 2 Material and methods

### 2.1 Study site and sediment sampling

Samples were taken during the R/V *Meteor* cruise M92 between 5 January and 3 February 2013 along a depth transect off the Peruvian coast from the shelf (~ 70 m) to the continental margin (~ 1000 m). The transect was located in the central part of the ESP-OMZ (Fuenzalida et al., 2009) at 12° S. Further hydrographic details on the study area can be found elsewhere (Dale et al., 2015).

Sediment cores for the determination of near-surface methanogenesis were collected at six stations along the depth transect at 70, 145, 253, 407, 776 and 1024 m water depth (Fig. 1), using a multiple corer with a mounted camera (TV-MUC). The MUC held seven cores (length: 60 cm, inner diameter: 10 cm) and covered an area of ~ 1 m<sup>2</sup>. If necessary, a second MUC was deployed at the same station, thus sediment cores could originate from different MUC casts. Station numbers were assigned in accordance with Dale et al. (2015). After retrieval, sediment cores were transferred to a ~ 9 °C cold room and processed at the same day.

In addition to the MUC, a gravity corer was deployed at two stations (78 and 407 m) for determining deep methanogenesis. The total core length was 400 and 206 cm, respectively. The gravity corer was equipped with a 260 kg weight and a 5 m steel barrel (diameter: 14 cm). The replaceable core liner (PVC, diameter: 12.5 cm) was housed within the barrel and fixed with a core catcher. After retrieval, sediment cores from the gravity corer were sliced into 1 m sections, capped on both sides, and brought to the cold room (4 °C) for further processing. Relevant station details for MUC and gravity cores are summarized in Table 1.

### 2.2 Water column sampling

CTD/Rosette water column casts were conducted at the same station as sediment coring (for details see Table 1). Temperature and oxygen data were taken from Dale et al. (2015).

For the analysis of methane concentrations in the bottom-near water, water was sampled ca. 1.5 m above the seafloor from 10 L Niskin bottles mounted on the CTD/Rosette. The collected water was filled bubble-free into 60 mL vials (triplicates), each vial containing three pellets of sodium hydroxide (NaOH, ~ 0.3 M per vial) to stop microbial activity and force dissolved gas into the headspace. After closing the vials with a butyl rubber stopper and a crimp seal, 10 mL of water was removed with a N<sub>2</sub>-flushed 10 mL syringe and replaced with N<sub>2</sub> gas from a second syringe to create a headspace in the sampling vials. Samples were stored and transported at room temperature until further processing.

In the home laboratory, 100 µL of the headspace volume was injected into a Shimadzu GC-2014 gas chromatograph equipped with a flame ionization detector and a HaySep-T 100/120 column (Length 3 m, diameter: 2 mm). Gases were separated isothermally at 75 °C with helium carrier gas. Methane concentrations were calibrated against methane standards (Scotty gases). The detection limit was 0.1 ppm with a precision of 2 %.

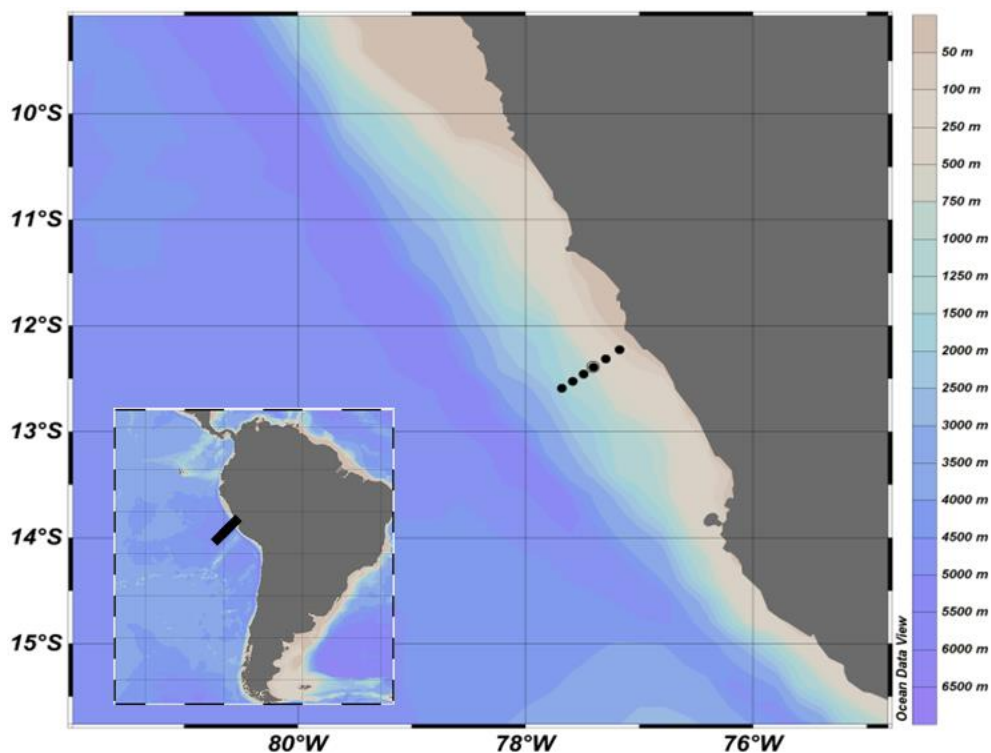
### 2.3 Porewater geochemistry

Porewater sampling for MUC cores has been previously described by Dale et al. (2015). In short, one MUC core per station was subsampled in an argon-filled glove bag to preserve redox-sensitive constituents.

The gravity cores at St. 1 (78 m) and St. 8 (407 m) were subsampled at 10–12 different sediment depths (depending on core length) resulting in depth intervals of 20–33 cm. Before sampling, the plastic core liner was cut open with an electric saw at the specific depths. Porewater was extracted by using anoxic (flushed with argon), wetted rhizons (Rhizosphere Research Products, Seeberg-Elverfeldt et al., 2005).

Sulfate concentrations were determined by ion chromatography (Methrom 761) as described previously by Dale et al. (2015).

For DIC analysis, 1.8 mL of porewater was transferred into a 2 mL glass vial, fixed with 10 µL saturated mercury chloride solution and crimp sealed. Samples were stored at 4 °C until further processing in the home laboratory. DIC concentration was determined as CO<sub>2</sub> with a multi N/C 2100 analyzer (Analytik Jena) following the manufacturer's instructions. Therefore the sample was acidified with phosphoric acid and the outgassing CO<sub>2</sub> was measured. The detection limit was 20 µM with a precision of 2–3 %.



**Figure 1.** Location of sampling sites off Peru along the depth transect at 12° S. Source: Schlitzer, R., Ocean Data View, <http://odv.awi.de>, 2014.

#### 2.4 Sediment porosity and particulate organic carbon/nitrogen

Methodology and data for porosity, particulate organic carbon (POC) and particulate organic nitrogen (PON) have been previously described by Dale et al. (2015).

In short, wet sediment samples were taken from the pore-water MUC core and the gravity cores for determination of porosity from the weight difference of wet and freeze-dried sediment. POC and PON were analyzed with a Carlo Erba element analyzer (NA 1500). Ratios of POC : PON were calculated by division.

#### 2.5 Sediment methane

For sediment methane concentration, one MUC core per station was sliced in 2 cm intervals until 20 cm depth, followed by 5 cm intervals until the end of the core (maximum depth = 48 cm). Gravity cores were subsampled according to the above scheme (see Sect. 2.3). From each sampled sediment layer, 2 cm<sup>-3</sup> sediment were transferred into a 15 mL serum glass vial containing 5 mL of NaOH (2.5 % *w/w*). The vial was closed with a butyl stopper, crimp sealed and shaken thoroughly to stop microbial activity and to force all methane into the headspace. Vials were stored upside down at room temperature until measurement in the home laboratory.

Sediment methane concentration was determined by injecting 0.1 mL of headspace volume into a Shimadzu GC-2014 gas chromatograph as described under Sect. 2.2.

#### 2.6 Net methanogenesis activity in MUC cores

Sediment from MUC cores was used to determine net methanogenesis, which is defined as the sum of total methane production and consumption, including all available methanogenic substrates in the sediment. Net methanogenesis was determined by measuring the linear increase of methane concentration in the headspace of closed incubation vials over time. Therefore, one MUC core per station was sliced into 5 cm intervals, transferring 10 cm<sup>-3</sup> of sediment in triplicates into N<sub>2</sub>-flushed 60 mL serum glass vials. The sediment core lengths ranged from 25 up to 48 cm, resulting in a maximum of 10 depth intervals. Then 10 mL of anoxic deep water overlying each MUC core was added to the vial and the slurry was mixed under a constant N<sub>2</sub> stream (Hungate, 1950) before being sealed with a butyl rubber stopper and crimped. The sediment slurry was repeatedly flushed with N<sub>2</sub> through the stopper to ensure fully anoxic conditions. The vials were incubated in the dark and at 9 °C, which reflected the average in situ temperature along the depth transect (see Table 1). The first gas chromatographic measurement was done directly after preparation of the vials, by injecting 100 µL of headspace sample into the

**Table 1.** Stations, instruments, chemical/physical parameters in the bottom-near water, and analyses applied to samples along the depth transect on the Peruvian margin (12° S). For abbreviations see footnote.

Station no.	Instrument	Latitude (S)	Longitude (W)	Water depth (m)	O <sub>2</sub> (μM)	Temp. (°C)	CH <sub>4</sub> (nM)	Type of analysis
1	MUC 13	12°13.492	77°10.511	70				All
	MUC 38	12°13.517	77°10.084	70				SE
	GC 8	12°14.500	77°9.611	78				GC-All
	CTD 9	12°13.535	77°10.522	73	bdl	14	38.6	WC
4	MUC 10	12°18.704	77°17.790	145				All
	CTD 14	12°18.697	77°18.004	145	bdl	13.4	24.4	WC
6	MUC 5	12°23.321	77°24.176	253				Gas+PW
	MUC 6	12°23.322	77°24.181	253				nMG
	CTD 6	12°24.904	77°26.314	305	bdl	12	79.6	WC
8	MUC 23	12°27.198	77°29.497	407				Gas+ PW
	MUC 24	12°27.197	77°29.497	407				nMG
	GC 3	12°27.192	77°29.491	407				GC-All
	CTD 37	12°29.502	77°29.502	407	bdl	10.6	7.3	WC
9	MUC 17	12°31.374	77°35.183	770				Gas+ PW
	MUC 18	12°31.373	77°35.184	770				nMG
	CTD 27	12°31.327	77°35.265	770	19	5.5	8.4	WC
10	MUC 28	12°35.377	77°40.975	1024				Gas+ PW
	MUC 29	12°35.377	77°40.976	1024				nMG
	CTD 11	12°34.863	77°38.954	1010	53	4.4	3.9	WC

MUC = multicorer, GC = gravity corer, CTD = CTD/Rosette, bdl = below detection limit (5 μM), All = methane gas analysis, porewater analysis, net methanogenesis analysis, SE = slurry experiment, GC-All = analysis for gravity cores including methane gas analysis, porewater analysis, hydrogenotrophic methanogenesis analysis, WC = water column analyses, Gas = methane gas analysis, PW = porewater analysis, nMG = net methanogenesis analysis.

gas chromatograph. The on-board Hewlett Packard 5890 gas chromatograph was equipped with a flame ionization detector and a HaySep-T 100/120 column (Length 3 m, diameter: 2 mm). Gases were separated isothermally at 75 °C with helium carrier gas. Methane concentrations were calibrated against methane standards. The detection limit was 1 ppm with a precision of < 5 %, Measurements were done in 2–4-day intervals over a total incubation time of ~ 2 weeks.

## 2.7 Potential non-competitive and competitive methanogenesis in sediment slurries from MUC cores

Sediment slurry experiments were conducted with sediment from St. 1 (70 m) to examine the interaction between sulfate reduction and methanogenesis, as this station revealed the highest microbial activity of sulfate reduction and methanogenesis. On board, the sediment core was sliced in 5 cm intervals. Sediment from the 0–5 cm interval and the 20–25 cm interval was transferred into 250 mL glass bottles, which were then closed without headspace (filled to top) with a butyl rubber stopper and screw cap. Until further treatment, sediment was stored at 4 °C on board and later in a 1 °C cold room on shore.

Approximately 6 months after the cruise, sediment slurries from both depth intervals were prepared by mixing 5 mL sediment in a 1 : 1 ratio with artificial, fully marine seawater (Widdel and Bak, 1992) before further manipulations.

In total, three different treatments, each in triplicate, were prepared per depth: (1) sulfate-rich (28 mM), serving as a control (2) sulfate-rich plus molybdate (22 mM) from now on referred to as molybdate treatment, and (3) sulfate-rich plus methanol (10 mM) from now on referred to as methanol treatment.

Molybdate was used as an enzymatic inhibitor for sulfate reduction (Oremland and Capone, 1988). Methanol is a known non-competitive substrate used by methanogens, but not by sulfate reducers (Oremland and Polcin, 1982), which makes it suitable to examine non-competitive methanogenesis.

The sediment slurries were incubated at 9 °C in the dark for 23 days and headspace concentration of methane was measured repeatedly over time on a gas chromatograph. Therefore, 100 μL of headspace was removed from the gas vials and injected into a Shimadzu gas chromatograph (GC-2014) equipped with a methanizer (inactive), a packed Haysep-D column and a flame ionization detector. The column temperature was 80 °C and the helium flow was

set to  $12 \text{ mL min}^{-1}$ . Methane concentrations were measured against methane standards. The detection limit was 0.1 ppm with a precision of  $< 5 \%$ . Rates were determined from the linear increase of methane concentration over time. Due to differences in the linear increase between the three treatments, rates were determined at two different time points: the first period of incubation includes the starting point (day 0) until day 5, the second period includes day 8 to day 23 (Supplement Fig. S1).

Student's  $t$  test (independent, two-tailed,  $\alpha = 0.05$ ) was applied to detect significant differences between the three different treatments.

## 2.8 Gross hydrogenotrophic methanogenesis activity in gravity cores

For the determination of surface to deep methanogenesis activity in gravity cores the radiotracer technique using  $^{14}\text{C}$  bicarbonate was applied (Jørgensen, 1978). With this method only hydrogenotrophic methanogenesis from  $\text{CO}_2/\text{H}_2$  can be determined, which is the expected main pathway in deeper sediment layers.

Sampled sediment depths were according to the sampling scheme described under Sect. 2.3. Circa  $5 \text{ cm}^{-3}$  of sediment was sampled in triplicates into glass tubes equipped with syringe plungers and then sealed headspace-free with butyl rubber stoppers. Then,  $^{14}\text{C}$ -bicarbonate tracer (dissolved in water,  $\text{pH} = 8\text{--}9$ , injection volume  $6 \mu\text{L}$ , activity  $222 \text{ kBq}$ , specific activity  $1.85\text{--}2.22 \text{ GBq mmol}^{-1}$ ) was injected through the stopper. The vials were incubated for 48 h at  $9^\circ\text{C}$  before the reaction was stopped by transferring the sediment into 50 mL glass vials filled with 20 mL NaOH (2.5 %), closed with butyl rubber stoppers and shaken thoroughly. Five controls were produced from various sediment depths by injecting the radiotracer directly into the NaOH with sediment.

In the home laboratory,  $^{14}\text{C}$ -methane production was determined with the slightly modified method of Treude et al. (2005a) used for the determination of anaerobic oxidation of methane. The method was identical, except that no unlabeled methane was determined by gas chromatography. Instead, DIC values were used to calculate hydrogenotrophic methane production (=  $\text{CO}_2$  reduction):

$$\text{MG rate} = \frac{^{14}\text{CH}_4 \times [\text{DIC}]}{(^{14}\text{CH}_4 + ^{14}\text{C-DIC}) \times t} \quad (1)$$

The methanogenesis rate (MG rate) is expressed in  $\text{nmol CH}_4 \text{ cm}^{-3} \text{ sediment d}^{-1}$ ,  $^{14}\text{CH}_4$  is the activity of produced  $^{14}\text{CH}_4$ ,  $^{14}\text{C-DIC}$  is the activity of residual radioactive dissolved organic carbon ( $\text{DIC} = \text{CO}_2 + \text{HCO}_3^- + \text{CO}_3^{2-}$ ),  $[\text{DIC}]$  is the concentration of dissolved organic carbon in  $\text{nmol cm}^{-3}$  sediment, and  $t$  is the incubation time in days.

## 2.9 Sulfate reduction in MUC cores

One MUC core per station was used for the determination of sulfate reduction. First, two replicate push cores (length 30 cm, inner diameter 2.6 cm) were subsampled from one MUC core. The actual core length varied from 23–25 cm b.s.f. total length. Then,  $6 \mu\text{L}$  ( $\sim 150 \text{ kBq}$ ) of carrier-free  $^{35}\text{SO}_4^{2-}$  radiotracer (dissolved in water, specific activity  $37 \text{ TBq mmol}^{-1}$ ) was injected into the replicate push cores in 1 cm intervals according to the whole-core injection method of Jørgensen (1978). Push cores were incubated for ca. 12 h at  $9^\circ\text{C}$ . After incubation, bacterial activity was stopped by slicing the push core into 1 cm intervals and transferring each sediment layer into 50 mL plastic centrifuge tubes filled with 20 mL zinc acetate (20 %  $w/w$ ). Controls were done in triplicate from different depths. Here, the sediment was first fixed with zinc acetate before adding the tracer. Rates for sulfate reduction were determined using the cold chromium distillation procedure according to Kallmeyer et al. (2004).

The yielded sulfate reduction rates have to be treated with caution, due to long (up to three half-life times of  $^{35}\text{S}$ ) and unfrozen storage. Storage of sulfate reduction samples without freezing has recently been shown to result in the re-oxidation of  $^{35}\text{S}$  sulfides, which results in an underestimation of sulfate reduction rates (Røy et al., 2014). During this reaction, zinc sulfide ( $\text{Zn}^{35}\text{S}$ ) and iron sulfide ( $\text{Fe}^{35}\text{S}$ ) are re-oxidized to sulfate by reactive  $\text{Fe(III)}$ , which originates from the reaction of  $\text{Fe}^{2+}$  with oxygen.  $\text{Fe}^{2+}$  is released during the gradual conversion of  $\text{FeS}$  to  $\text{ZnS}$ , which has the lower solubility product. Still, we do trust the relative distribution of activity along depth profiles and consider a potential underestimation of absolute rates.

## 3 Results

### 3.1 Water column oxygen and methane concentration

Dissolved oxygen in the bottom water was below detection limit from St. 1 (70 m) to St. 8 (407 m), subsequently increasing with water depth to  $53 \mu\text{M}$  at the deepest site (see Table 1 and Dale et al., 2015). At the shallowest St. 1 (70 m) the water was turbid and smelled of sulfide.

Dissolved methane concentrations in the bottom water were high on the shelf (St. 1–6, 70–253 m) and 10-fold lower at the deeper sites (St. 8–10, 407–1024 m; Table 1). The highest measured methane concentration was detected at St. 6 (253 m,  $\sim 80 \text{ nM}$ ) and lowest concentrations were detected at St. 10 (1024 m,  $\sim 4 \text{ nM}$ ).

### 3.2 Sediment core description

A detailed sediment description for the porewater geochemistry cores has been already published in detail by Dale et al. (2015). In short, sediments revealed a gray color with a black surface layer at St. 1 (70 m), a dark olive green color

at St. 4–8 (145–407 m), and a green-brown color at St. 9 and 10 (770–1024 m). Sediment texture was soft and fluffy at St. 1–6 (70–253 m), and was less soft at the deeper sites. St. 8 (407 m) revealed a fluffy surface layer followed by a dense clay layer >2 cm b.s.f. sediment depth. In addition, phosphorite nodules were found at the sediment surface (0–2 cm b.s.f.) of St. 8 (407 m).

Mats of the sulfur-oxidizing bacteria *Thioploca* spp. (Gallardo, 1977) were visible at the sediment surface at St. 1–6 (70–253 m), with the densest mat at St. 1 (70 m) continuously decreasing with increasing water depth. Sheaths of *Thioploca* were visible until 20–30 cm b.s.f. at St. 1, 4 and 6 (70–253 m).

Foraminifera could be observed at the sediment surface of St. 8 (407 m), St. 9 (770 m) and St. 10 (1024 m). St. 8 (407 m) showed a thick layer of foraminifera ooze on the sediment surface (0–3 cm b.s.f.) while St. 9 (770 m) and St. 10 (1024 m) showed only scattered foraminifera at the sediment surface (0–5 cm b.s.f.).

Macrofauna (large polychaetes, oligochaetes, ophiuroids) were restricted to the sites below the OMZ at St. 9 (770 m) and St. 10 (1024 m), where deep waters were oxygenated. However, small snails (~1 cm) were observed at St. 8 (407 m).

### 3.3 Geochemical parameters in MUC cores

Porewater and solid phase geochemistry of sediments retrieved by the MUC cores are shown in Fig. 2. Surface sediment (0–0.5 cm b.s.f.) POC content increased along the continental shelf from 1.6 wt % at the shallow St. 1 (70 m) to a maximum of 15 wt % at St. 8 (253 m). Surface POC content decreased again with increasing water depth showing the lowest POC content at St. 10 (1024 m, 2 wt %). While POC content showed more or less stable profiles throughout the sediment core at St. 1 (70 m, around 3 wt %), St. 9 (770 m, around 4 wt %) and St. 10 (1024 m, around 3 wt %), POC content was stable only in the upper ~10 cm b.s.f. at St. 4 (150 m, around 10 wt %) and St. 6 (253 m, around 15 wt %), followed by a decrease until the deepest sampled depth (2 and 9 wt %, respectively). At St. 8 (407 m), POC content increased with sediment depth below 3 cm b.s.f. (from 4 to 9 wt %), which consisted of dense clay (see above). In the upper 3 cm b.s.f., POC decreased from ~7 to ~4 wt %, which was the sediment layer with a more fluffy appearance.

The sediment surface C/N ratio was lowest at St. 1 (70 m, 6.2) and increased along the continental shelf showing the highest surface C/N ratio at St. 10 (1024 m, 11). St. 8 (407 m) was exceptional, as it showed slightly lower surface C/N ratio (8) as at St. 6 (253 m, 9). St. 8 (407 m) was also the only site showing an increase of four units in the upper 0–5 cm b.s.f., followed by stable ratios around 12 throughout the rest of the core. St. 1 and 4 (70 and 145 m) showed shallower increases in C/N ratio in the upper ~2 cm b.s.f. and upper 1 cm b.s.f., respectively, followed by stable ratios

around 10 until the bottom of the core. At St. 9 and 10 (770 and 1024 m), C/N ratios ranged around 11 and 12, respectively.

The highest increase in methane concentration was observed at St. 1 (70 m). Here, methane increased linearly from the surface (1  $\mu\text{M}$ ) to the bottom of the core (100  $\mu\text{M}$ ). All other stations showed either no clear trend (St. 4 = 145 m) or only slight methane increases with depth. At St. 9 (770 m), even a decrease in methane concentration was observed from the surface to the bottom of core.

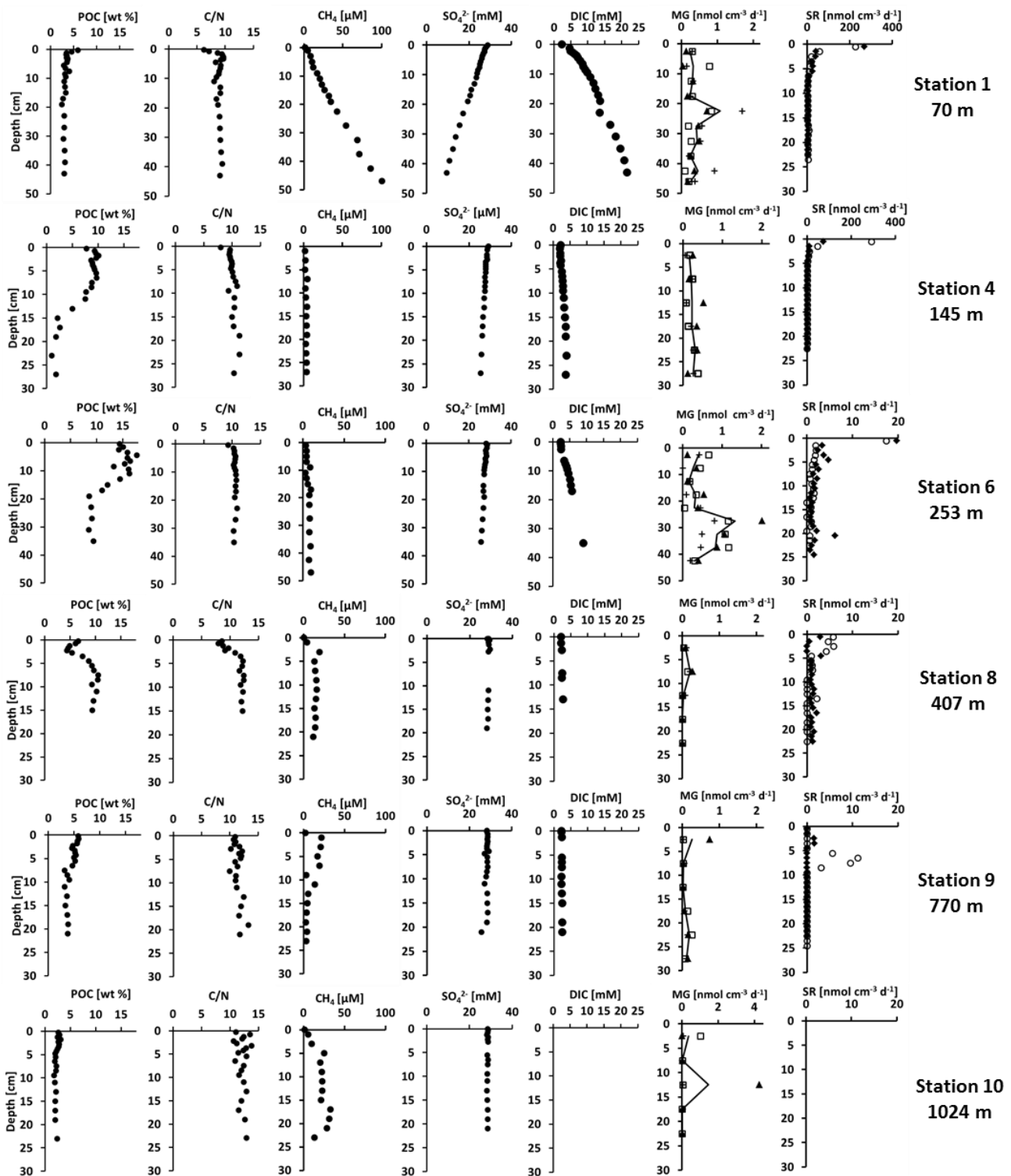
Besides St. 1 (70 m), which showed a strong decrease in sulfate ( $\text{SO}_4^{2-}$ ) concentration with depth from about 28 mM at the top to about 9 mM at the bottom of the core (43 cm b.s.f.), all other stations showed  $\text{SO}_4^{2-}$  concentrations >25 mM throughout the cores. At St. 4, 6 and 9 (145, 253, 770 m),  $\text{SO}_4^{2-}$  showed very slight decrease with depth from about 28 mM at the top to about 25 mM at the bottom of the core. Porewater  $\text{SO}_4^{2-}$  concentrations were stable around 28 mM throughout the core at St. 8 and 10 (407 and 1024 m).

Dissolved inorganic carbon (DIC) concentration increased with depth at St. 1–6 (70–253 m). St. 1 (70 m) showed the steepest increase with depth, showing the lowest DIC concentration at the top (2.3 mM) and the highest at the deepest sampled depth (21.6 mM). At St. 4 (153 m), maximum concentration was reached at ~23 cm b.s.f. with 4 mM. St. 6 (253 m) showed maximum concentration at the deepest sampled depth with 9 mM. St. 8 and 9 (407 and 770 m) showed stable DIC concentrations around 2.3 mM throughout the core. No DIC data were available for St. 10 (1024 m).

### 3.4 Net methanogenesis and gross sulfate reduction in MUC cores

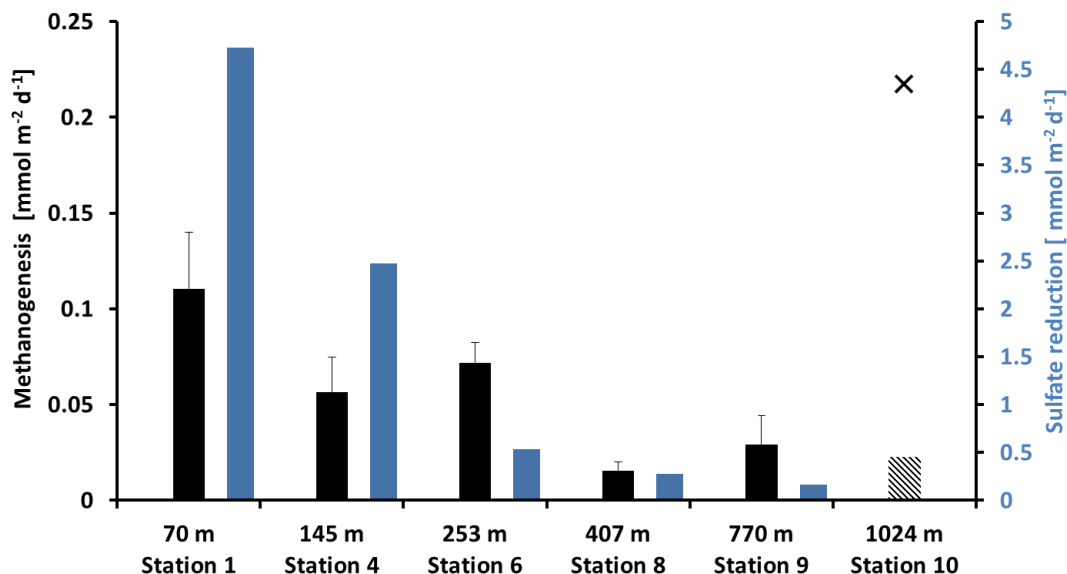
Maximum net methanogenesis rates (Fig. 2) were detected at St. 1 (70 m,  $1.1 \pm 0.5 \text{ nmol cm}^{-3} \text{ d}^{-1}$ , 20–25 cm b.s.f.) and St. 6 (253 m,  $1.3 \pm 0.65 \text{ nmol cm}^{-3} \text{ d}^{-1}$ , 25–30 cm b.s.f.). At all other stations, methanogenesis was mostly below  $0.5 \text{ nmol cm}^{-3} \text{ d}^{-1}$  throughout the cores. St. 8 (407 m) showed methanogenesis activity only in the top 10 cm b.s.f. with the maximum at 5–10 cm b.s.f. ( $0.2 \pm 0.5 \text{ nmol cm}^{-3} \text{ d}^{-1}$ ). At St. 9 and 10 (770 and 1024 m), maximum methanogenesis activity was found in the surface layer (0–5 cm b.s.f.) with  $0.3 \pm 0.4$  and  $0.4 \pm 0.6 \text{ nmol cm}^{-3} \text{ d}^{-1}$ , respectively. St. 10 (1024 m) also showed high average methanogenesis at 10–15 cm b.s.f. ( $1.5 \pm 2.5 \text{ nmol cm}^{-3} \text{ d}^{-1}$ ), which was caused by a single high replicate ( $4.3 \text{ nmol cm}^{-3} \text{ d}^{-1}$ ). In the following (e.g., integration of rates) we will exclude this single high replicate, which will be further elaborated in the discussion.

At all stations beside St. 9 (770 m), sulfate reduction activity was highest in the 0–1 cm b.s.f. horizon, followed by a sharp decrease in activity of 20–90 % in the subsequent 1–2 cm b.s.f. horizon. Highest measured rates at 0–1 cm b.s.f. were observed at St. 4 (145 m,  $290 \text{ nmol cm}^{-3} \text{ d}^{-1}$ ), followed by St. 1 (70 m,  $270 \text{ nmol cm}^{-3} \text{ d}^{-1}$ ). Surface (0–1 cm b.s.f.)



**Figure 2.** Profiles of particulate organic carbon (POC), C/N ratio, methane ( $\text{CH}_4$ ), sulfate ( $\text{SO}_4^{2-}$ ), DIC (dissolved inorganic carbon), net methanogenesis (MG) rates and sulfate reduction (SR) rates in the MUC cores along the depth transect. For MG, triplicates (symbols) and mean (solid line) are shown. For SR, duplicates are shown. Data points from the overlaying water in the MUC core (OLW) are set to 0 cm. Note deviant scale dimension for MG at St. 6 and for SR at St. 1 and 2.





**Figure 3.** Integrated methanogenesis and sulfate reduction rates (0–25 cm) along the depth transect. For methanogenesis rates (black bars), average values are shown with standard deviation. Note for St. 10 a mean from two replicates is shown without standard deviation (pattern-filled bar) and the outlier is shown separately (cross). For sulfate reduction rates (blue bars), means from two replicates are shown without standard deviation.

sulfate reduction activity decreased from St. 4 (145 m) to St. 8 (407 m) with concomitant increase in water depth. St. 9 (770 m) was the only site without a surface sulfate reduction maximum. Here, highest rates were found at 7 cm b.s.f. ( $11.2 \text{ nmol cm}^{-3} \text{ d}^{-1}$ ).

St. 6, 8 and 9 (253, 407, and 770 m) showed a second but smaller maximum of sulfate reduction activity. At St. 6 (253 m), this second maximum was situated at 20.5 cm b.s.f. ( $6.2 \text{ nmol cm}^{-3} \text{ d}^{-1}$ ). St. 8 and 9 (407 and 770 m) showed additional maxima at 4.5 cm b.s.f. ( $3.1 \text{ nmol cm}^{-3} \text{ d}^{-1}$ ) and 2.5 cm b.s.f. ( $1.5 \text{ nmol cm}^{-3} \text{ d}^{-1}$ ), respectively. At St. 9 (770 m), sulfate reduction activity was not detectable at most depths > 10 cm b.s.f.. At St. 10 (1024 m), no sulfate reduction activity was detectable throughout the entire core. At St. 9 and 10 (770 and 1024 m) we cannot exclude that sulfate reduction was present but undetectable due to long, unfrozen storage of the samples (see Sect. 2.7).

Figure 3 shows an overview of integrated methanogenesis and sulfate reduction rates (over the upper 0–25 cm) along the depth transect on the Peruvian margin. Highest integrated surface methanogenesis activity was detected on the shelf (70, 145 and 253 m) with  $0.1 \pm 0.03$ ,  $0.06 \pm 0.02$ , and  $0.07 \pm 0.01 \text{ mmol m}^{-2} \text{ d}^{-1}$ , respectively. St. 8 (407 m) revealed the lowest integrated methanogenesis rate of all sites ( $0.02 \pm 0.00 \text{ mmol m}^{-2} \text{ d}^{-1}$ ). St. 9 (770 m) and St. 10 (1024 m) showed integrated methanogenesis activity around  $0.03 \pm 0.02 \text{ mmol m}^{-2} \text{ d}^{-1}$ , respectively.

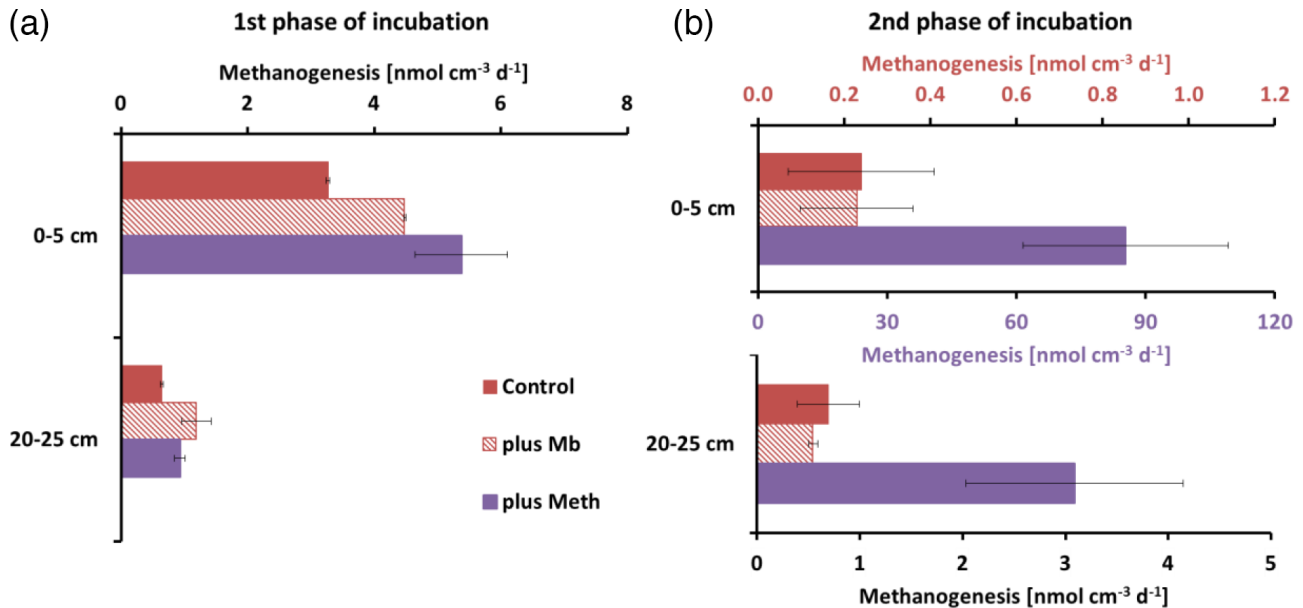
Integrated sulfate reduction activity decreased along the continental margin with increasing water depth, revealing the highest activity at the St. 1 (70 m,  $4.7 \text{ mmol m}^{-2} \text{ d}^{-1}$ ) and the

lowest activity at St. 9 (770 m,  $0.2 \text{ mmol m}^{-2} \text{ d}^{-1}$ ). Please note again, that integrated sulfate reduction rates are probably underestimated due to long, unfrozen storage of the samples (see Sect. 2.7).

### 3.5 Potential competitive and non-competitive methanogenesis in sediment slurries from MUC cores

Results from the sediment slurry experiments, in which we added either the sulfate reduction inhibitor molybdate, the non-competitive substrate methanol, or no additives (control), are shown in Fig. 4. During the first phase of incubation, all three treatments showed rates within the same order of magnitude. Nevertheless, potential methanogenesis rates were significantly higher ( $p < 0.05$ ) in all treatments in the shallow sediment horizon (0–5 cm b.s.f.) compared to the deep horizon (20–25 cm b.s.f.). In addition, potential methanogenesis was always significantly higher in the molybdate and methanol treatment compared to the control.

During the second phase of the incubation (days 8–23), potential methanogenesis showed a different pattern. Rates in the methanol treatment were 350 and 4 times higher compared to the control and molybdate treatment in the 0–5 cm horizon and the 20–25 cm horizon, respectively ( $p < 0.05$ ). Control and molybdate treatments showed no significant difference ( $p > 0.05$ ) in the shallow and deep horizon.



**Figure 4.** Potential methanogenesis rates in sediment slurry experiments from the two sediment intervals (0–5 and 20–25 cm) at St. 1 (70 m). The first phase of the incubation shows rates calculated from day 0–5 (a), while the second phase of the incubation summarizes the rates from day 8–23 (b). “Control” is the treatment with sulfate-rich (28 mM) artificial seawater medium, “plus Mb” is the treatment with sulfate-rich artificial seawater medium plus molybdate (Mb, 22 mM), and “plus Meth” is defined as the treatment with sulfate-rich artificial seawater medium plus methanol (Meth, 10 mM). Per treatment, average values are shown with standard deviation. Please note the split-up in the diagram in (b) and the different  $x$  axis for methanogenesis.

### 3.6 Geochemical parameters and gross hydrogenotrophic methanogenesis activity in gravity cores

At the shallow St. 1 (78 m), POC concentration slightly decreased with depth, from  $\sim 4$  wt % at the surface to about 2–3 wt % at the bottom of the core (385 cm b.s.f., Fig. 5). At St. 8 (407 m), POC concentrations were slightly higher with values ranging around 8–9 wt % in the upper 120 cm b.s.f., and then decreasing with depth. The C/N ratio at St. 1 (78 m) remained around 10 throughout the core, while it showed slightly higher values around 12 throughout the core at St. 8 (407 m).

At St. 1 (78 m), the methane concentration increased with depth from 0.1 mM at the surface to the highest measured concentration at 165 cm b.s.f. ( $\sim 5$  mM), followed by a decrease to  $\sim 2$  mM at 198 cm b.s.f. Methane concentration stayed around 2 mM until the deepest measured depth (385 cm b.s.f.).

Methane concentrations at St. 8 (407 m) ranged from 14 to 17  $\mu$ M in the upper 120 cm b.s.f., then increased to a maximum of 36  $\mu$ M at 180 cm b.s.f., followed by a decrease to 28  $\mu$ M at the deepest sampled depth (195 cm b.s.f.).

$\text{SO}_4^{2-}$  concentration at St. 1 (78 m) decreased with depth with the highest concentration (10 mM) at the shallowest measured sediment depth (33 cm b.s.f.) and the lowest concentration at 350 cm b.s.f. (0.16 mM). At St. 8 (407 m),  $\text{SO}_4^{2-}$

concentration decreased slightly from  $\sim 28$  mM at the shallowest measured sediment depth (20 cm b.s.f.) to  $\sim 24$  mM at 145 cm b.s.f., followed by stable concentrations around 25 mM until the bottom of the core.

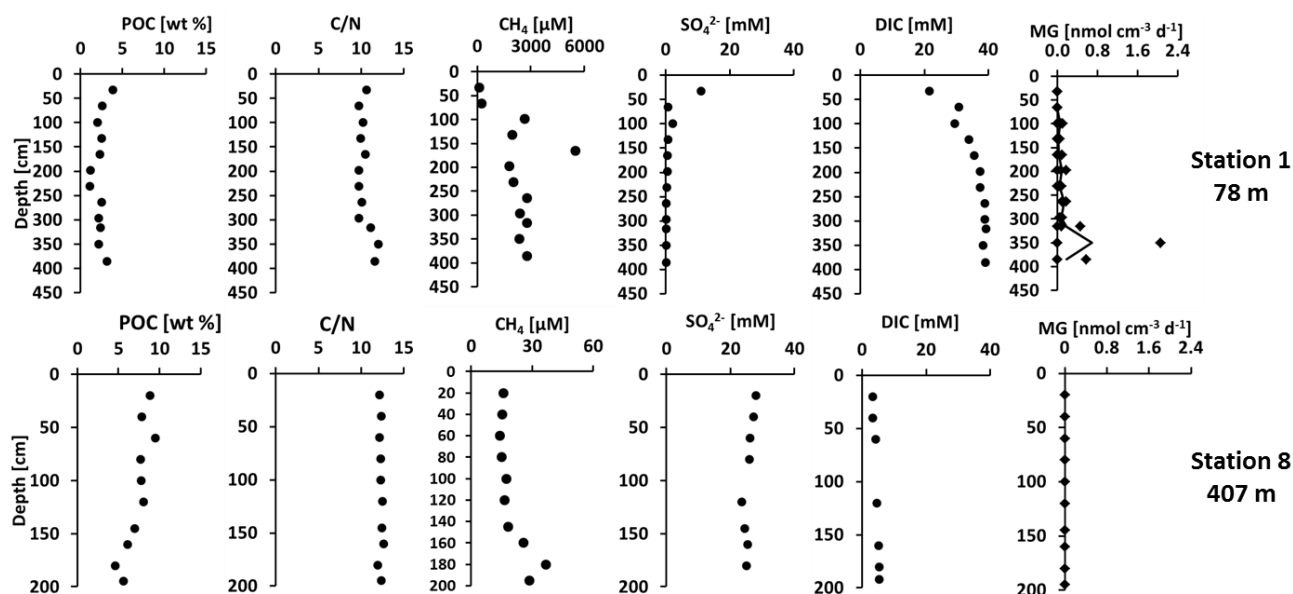
DIC concentrations were 5–8 times higher at St. 1 (78 m) compared to St. 8 (407 m) and increased with sediment depth from  $\sim 21$  at 33 cm b.s.f. to  $\sim 39$  mM at 385 cm b.s.f. DIC concentrations at St. 8 (407 m) could only be measured at distinct sediment depths due to limited amounts of porewater but still revealed a slight increase with sediment depth (from  $\sim 3$  to  $\sim 5$  mM).

Hydrogenotrophic methanogenesis at St. 1 (78 m) was present but low below 66 cm b.s.f. until it reached a peak between 300 and 400 cm b.s.f. ( $0.7 \text{ nmol cm}^{-3} \text{ d}^{-1}$ ). In contrast, no hydrogenotrophic methanogenesis activity was detected at St. 8 (407 m).

## 4 Discussion

### 4.1 Concurrent activity of methanogenesis and sulfate reduction in surface sediments

Before we discuss the distribution of methanogenesis in the collected sediment cores, it has to be pointed out that the top soft sediment layer (ca. 0–20 cm) of gravity cores is often disturbed or even lost during the coring procedure. Hence, surface parameters in the gravity cores should not



**Figure 5.** Profiles of particulate organic carbon (POC), C/N ratio, methane ( $\text{CH}_4$ ), sulfate ( $\text{SO}_4^{2-}$ ), dissolved inorganic carbon (DIC), and hydrogenotrophic methanogenesis (MG) rates in the gravity cores at two stations within the depth transect. For MG, triplicates (symbols) and mean (solid line) are shown.

be directly compared to the respective depth layers in MUC cores. According to this likely offset, we will use the term “surface methanogenesis/sediments” when referring to MUC cores and “deep methanogenesis/sediments” when referring to gravity cores.

We would further like the reader to keep in mind that we will compare two different types of rate determinations: radiotracer incubations of undisturbed sediments (deep hydrogenotrophic methanogenesis, surface sulfate reduction) and sediment slurry incubations (surface total methanogenesis). While the first method preserves the natural heterogeneity of the sediment, the latter homogenizes and dilutes sediment ingredients and organisms, which could have both negative and positive effects on the microbial activity. As we are mainly interested in the vertical distribution of these processes within the sediment, these comparisons are justifiable.

In the present study, methanogenesis and sulfate reduction co-occurred in surface sediments along the entire depth transect (70–1024 m) on the Peruvian margin ( $12^\circ\text{S}$ ). Methanogenesis activity was detected in sediment layers that revealed high porewater sulfate concentrations and sulfate reduction activity (besides St. 10, where sulfate reduction was undetectable). Even though absolute sulfate reduction rates were most likely underestimated, we trust relative distribution pattern in the sediment and along the continental margin.

As the competition between methanogens and sulfate reducers for  $\text{H}_2$  and acetate was probably never relieved, the detected surface methanogenesis was most likely based on non-competitive substrates such as methanol or methylated compounds including methylated amines or methylated sulfides (Oremland and Polcin, 1982; Oremland and Taylor,

1978; Kiene et al., 1986). Likewise, in a study off Chile (0–6 cm sediment depth, 87 m water depth), surface methanogenesis was found to be coupled to the non-competitive substrate trimethylamine, and not to  $\text{CO}_2/\text{H}_2$  or acetate, in sediments where sulfate and sulfate reduction was abundant (Ferdeman et al., 1997).

Non-competitive substrate utilization by methanogens in the present study was further confirmed by a significant increase of potential methanogenesis after the addition of methanol to sediment slurries from St. 1 (70 m) (Fig. 4b). The delayed response of methanogenesis after methanol addition (Supplement Fig. S1), however, suggests that the present microbial methanogenic community was not primarily feeding on methanol. Potentially, other non-competitive substrates like dimethyl sulfides were utilized predominantly. While most methylotrophic methanogens are able to use both methanol and methylated amines, growth on dimethyl sulfide appears to be restricted to only a few methylotrophic species (Oremland et al., 1989). Dimethyl sulfides could have accumulated during the long storage time ( $\sim 6$  months) before experimentation. Even though methylated sulfur compounds (e.g., dimethyl sulfide or methanethiol) are mainly produced by organisms in the marine photic zone (e.g., Andreae and Raemdonck, 1983), it was recently postulated that these compounds may also be generated through nucleophilic attack by sulfide on methyl groups in the sedimentary organic matter (Mitterer, 2010). As sulfate reduction was a predominant process in the sediment, it could have delivered sufficient sulfide to produce methylated sulfur compounds. Consequently, results from the sediment slurry experiments might not reflect the activity of the in situ methanogenic community as we

cannot exclude community shifts as a response to the availability of alternative substrates that were produced during the long storage.

The utilization of the competitive substrates  $H_2$  and acetate by the methanogens probably only occurs when sulfate reducers are inhibited. Accordingly, potential methanogenesis rates in the molybdate treatment of the sediment slurry experiment were significantly higher in the two studied horizons (0–5 and 20–25 cm b.s.f.) compared to the controls during the first phase of the incubation (days 0–5), indicating the usage of competitive substrate facilitated by the inhibition of sulfate reduction. However, in the second phase (days 8–23) of the incubation, rates were much lower in both the control and molybdate treatment and did not show significant differences in both horizons ( $p > 0.05$ ). In this second phase, methane production might have slowed down due to depletion of electron donors.

Hydrogenotrophic methanogenesis in the gravity core from St. 1 (78 m) showed no activity at depths where porewater sulfate concentrations were  $> 0.7$  mM. Instead activity peaked where porewater sulfate was lowest (0.16 mM at 350 cm b.s.f.), supporting the above conclusions regarding competition within the sulfate zone. The observation that sulfate was never completely depleted in the porewater until the bottom of the gravity core, in combination with an increase of iron (II) in the porewater at depths  $> 200$  cm b.s.f. (data not shown), hint to the presence of a cryptic sulfur cycle that is responsible for deep formation of sulfate (Holmkvist et al., 2011; Treude et al., 2014).

In comparison, surface net methanogenesis activity along the Peruvian margin was similar to activities found off Chile at 87 m water depth ( $0\text{--}0.6$  nmol  $cm^{-3} d^{-1}$ ) (Ferdelman et al., 1997). The slightly higher rates determined in our study (St.1 = 70 m;  $0.4\text{--}1.7$  nmol  $cm^{-3} d^{-1}$ ) could be related to different approaches, as our rates represent the sum of net methanogenesis from all available substrates in the sediment, while rates off Chile were based only on  $CO_2$ , acetate and trimethylamine utilization. Hence, total methanogenesis could have been easily underestimated, if methanogenesis was supplied by other substrates, which is not unlikely, as methylotrophic methanogens, which are able to use methanol or methylated amines, were the dominant type of methanogens in these sediments (Ferdelman et al., 1997). Interestingly, the authors detected a high number of acetogens, implying that acetogenesis competed for methylamines or other methylated compounds (Ferdelman et al., 1997). A competition with acetogens for methylated substrates is conceivable for our study, but would require further corroboration.

#### 4.2 Surface vs. deep methanogenesis

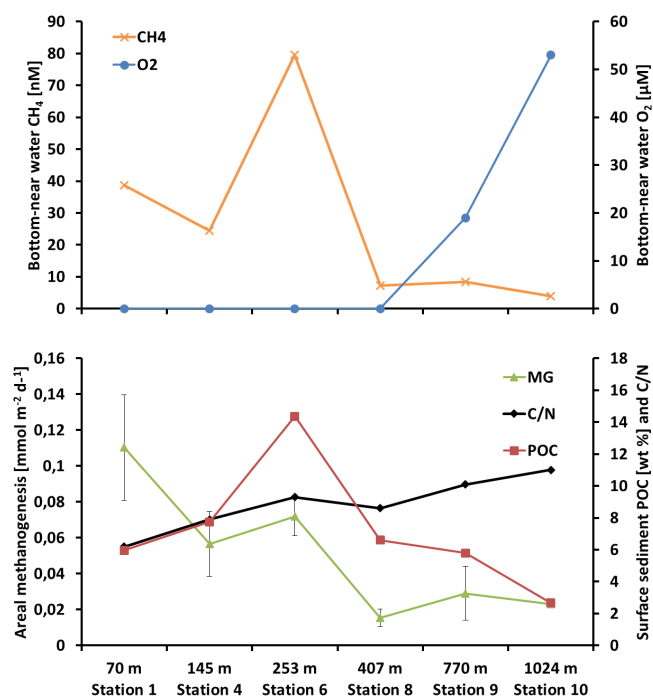
Maximum single net surface methanogenesis activities detected in our study ( $0.3\text{--}4.3$  nmol  $cm^{-3} d^{-1}$ ) were found to be at the very low end or even one order of mag-

nitude lower than organic-rich, sulfate-depleted sediments ( $9\text{--}37$  nmol  $cm^{-3} d^{-1}$ , 0–40 cm b.s.f.; Treude et al., 2005a,  $10\text{--}17$  nmol  $cm^{-3} d^{-1}$ , 0–30 cm b.s.f.; Schmaljohann, 1996;  $100\text{--}300$  nmol  $cm^{-3} d^{-1}$ , 0–30 cm b.s.f., Crill and Martens, 1983, 1986,  $100\text{--}400$  nmol  $cm^{-3} d^{-1}$ , 0–3 cm b.s.f.; Alperin et al., 1992). To estimate the overall relevance of surface methanogenesis within the sulfate zone compared to deep methane production, we estimated the deep methane production in our study and compiled an overview of published deep methane production data from the sulfate-free zone of organic-rich sediments (Table 2). For this comparison, the deep methane production was assumed to equal the flux of methane into the sulfate–methane transition zone (SMTZ), where it is consumed by anaerobic oxidation of methane (AOM). Within the SMTZ, both sulfate and methane are depleted steeply as a result of AOM, thus dividing the sulfate-reducing zone above from the methanogenic zone below. The SMTZ is the main niche for AOM in marine sediments, acting as an important filter for upwards migrating methane (Knittel and Boetius, 2009). The SMTZ can be found at decimeters to tens of meters below the seafloor, depending on the burial rate of reactive organic matter, the depth of the methane production zone, and the flux of methane and sulfate as well as their consumption rates (Knittel and Boetius, 2009).

In the present study, a SMTZ was only detected in the gravity core taken at St. 1 (78 m; Fig. 5), where it was located between 66 and 99 cm b.s.f. – i.e., below the penetration depth of the MUC cores. We estimated a methane flux (= deep methane production) into the SMTZ (from 99 to 66 cm b.s.f.) according to Iversen and Jørgensen (1993) using a seawater methane-diffusion coefficient from Schulz (2006) which was corrected for porosity resulting in a sediment-diffusion coefficient for methane of  $D_s = 1.325 \times 10^{-5} cm^2 s^{-1}$  at  $15^\circ C$ . The resulting deep methane production ( $0.8$  mmol  $m^{-2} d^{-1}$ ) was slightly higher (ratio of 0.13, surface vs. deep) but still in the same magnitude as the integrated surface methanogenesis at St. 1 (70 m;  $0.1$  mmol  $m^{-2} d^{-1}$ ). Compared to a different study from the Peruvian OMZ, the ratio between shallow ( $0.07$  to  $0.1$  mmol  $m^{-2} d^{-1}$ , this study) vs. deep ( $8.9 \times 10^{-8}$  to  $2.2 \times 10^{-7}$  mmol  $m^{-2} d^{-1}$ ; Arning et al., 2012) methanogenesis on the shelf (150–250 m) was  $3.2 \times 10^5$  to  $1.1 \times 10^6$ . Both examples highlight the significance of surface methanogenesis, especially on the Peruvian shelf. On the lower Peruvian slope ( $\sim 3800$  m water depth), deep methanogenesis increased (up to  $0.017$  mmol  $m^{-2} d^{-1}$ ; Arning et al., 2012). In contrast, surface methanogenesis at the deeper St. 10 (1024 m) was lower ( $0.02$  mmol  $m^{-2} d^{-1}$ ) compared to the shelf indicating a decreasing relevance of surface methanogenesis along the margin with increasing relevance of deep methanogenesis. The decrease of surface methanogenesis with increasing water depth might be correlated to the decreasing organic carbon content and freshness in the sediment (Fig. 6), as further discussed in Sect. 4.4.

**Table 2.** Comparison of deep methanogenesis in organic-rich sediments from different regions with surface methanogenesis ( $0.02\text{--}0.1\text{ mmol m}^{-2}\text{ d}^{-1}$ ) determined in the present study. The ratio range was achieved by dividing the lowest surface by the highest deep and the highest surface by the lowest deep methanogenic activity, respectively.

	Water depth (m)	Depth of SMTZ (m b.s.f.)	Methane flux into the SMTZ = integrated deep methanogenesis ( $\text{mmol m}^{-2}\text{ d}^{-1}$ )	Ratio between surface methanogenesis (present study) and deep methanogenesis	Reference
Namibia (SE Atlantic)	1312–2060	3–10	0.07–0.15	0.13–1.43	Niewöhner et al. (1998)
Eckernförde Bay (SW Baltic Sea)	25–28	0.5–1.5	0.66–1.88	0.01–0.15	Treude et al. (2005a)
Chile (SE Pacific)	797–2746	3–4	0.068–0.13	0.15–1.47	Treude et al. (2005b)
Limfjorden (North Sea)	7–10	1–1.5	0.076	0.03–1.32	Jørgensen and Parkes (2010)
Peru (SE Pacific)	150–3819	2–50	$2.2 \times 10^{-7}$ –0.017	$1.18\text{--}4.55 \times 10^5$	Arning et al. (2012)
Peru (SE Pacific)	70–1024	0.7–1	0.8	0.03–0.13	present study



**Figure 6.** Bottom-near water methane ( $\text{CH}_4$ ) and oxygen ( $\text{O}_2$ ) concentrations along the depth transect (above). Surface sediment particulate organic carbon (POC) content and C / N ratio together with integrated methanogenesis (MG) rates (0–25 cm b.s.f.) along the depth transect (below). For MG rates, averages are shown with standard deviation beside St. 10, where a mean from two replicates is shown (see text). Please note the secondary y axis.

In comparison with other organic-rich sediments (Table 2), surface methanogenesis off Peru was in the same order of magnitude as most reported deep methanogenesis (e.g., off Namibia, off Chile, Limfjorden). The only exception was Eckernförde Bay (Baltic Sea), where surface methanogenesis off Peru was less than 15 % of deep methanogenesis. Eckernförde Bay has a water depth of only  $\sim 30$  m with high carbon export, featuring extremely high methanogenesis activity below the SMTZ, causing supersaturation and methane gas ebullition (Whiticar, 2002; Treude et al., 2005a).

### 4.3 Potential consumption and emission of surface methane

Due to its closeness to the sediment–water interface, surface methanogenesis along the Peruvian margin could lead to methane emissions from the sediment into the water column. A short diffusion distance, especially in the topmost sediment layers, might facilitate a partial escape of methane from consumption by microbes. As surface methanogenesis decreased with water depth (Fig. 3), the methane emission potential appears to be highest on the shelf. Sediment methane concentrations in the 0–2 sediment horizon of all sites along the margin were always higher than bottom-near water methane concentrations ( $\sim 1.5$  m above seafloor; Table 1, Fig. 2), hinting towards an efflux of methane from the sediment. However, more precise profiling of methane at the sediment–water interface would be necessary to confirm this hypothesis. Still, most of the sediment methane profiles suggest methane consumption close to the seafloor to some extent, which would reduce the amount of emitted methane

(Fig. 2). AOM might act as an important methane filter at the sediment surface of the shelf stations, where anoxic conditions dominated, while aerobic oxidation might prevail at the deeper stations below the OMZ (St. 9 and 10). The presence of methane oxidation above the SMTZ of organic-rich sediments has been reported earlier (Treude et al., 2005a, b), and could indeed be fueled by surface methanogenesis. An immediate oxidation of the produced methane would explain why sediment methane profiles did not necessarily correlate with peaks in surface methanogenesis (see, e.g., St. 6, 253 m). The importance of AOM for the reduction of methane emissions from surface methanogenesis remains speculative, as explicit data are missing. On the basis of our findings, however, we suggest to consider surface methanogenesis as a possible driver for AOM above the SMTZ in earlier and future studies.

#### 4.4 Factors controlling methanogenesis along the Peruvian margin

For this discussion, we excluded the high integrated methane production observed in one of the replicates at station 10 (1024 m) as we do not think that the detected activity ( $0.23 \text{ mmol m}^{-2} \text{ d}^{-1}$ ) is representative for this deep site, especially as sediment POC content was lowest at station 10 compared to the other stations ( $< 4\%$ , Fig. 2). The outlier might have been caused by additional carbon sources in the sediment, e.g., from fecal pellets or organic carbon released from dead infauna, thus stimulating below-surface microbial activities during our incubations (Ziervogel et al., 2014; Bertics et al., 2013).

##### 4.4.1 Oxygen

Oxygen is an important controlling factor, as methanogenesis is an oxygen- and redox-sensitive process (Oremland, 1988). Some methanogens can tolerate oxygen exposure for several hours before they die, although no methane is produced in the presence of oxygen (Zinder, 1993).

Comparing integrated surface methanogenesis (over 0–25 cm b.s.f.) from the shallowest to the deepest station (Fig. 3), highest rates ( $> 0.05 \text{ mmol m}^{-2} \text{ d}^{-1}$ ) were detected on the shelf (St. 1, 4 and 6 = 70, 145, 253 m), where oxygen concentrations were below detection (Fig. 6), providing advantageous conditions for methanogenesis, particularly at the very sediment surface, where normally aerobic respiration dominates (Jørgensen, 2006). Below the OMZ, integrated methanogenesis decreased. Bioturbating macrofauna and megafauna (e.g., mussels, polychaetes, oligochaetes) were observed at these sites (St. 9 and 10, 770 and 1024 m) (Mosch et al., 2012), which could have transported oxygen into deeper sediment layer (Orsi et al., 1996), thus leading to less reduced conditions ( $> -200 \text{ mV}$ ) unsuitable for methanogens (Oremland, 1988). However, integrated methanogenesis was lowest at St. 8 (407 m), which still re-

vealed anoxic bottom water. Thus, oxygen might just be advantageous but not the driving factor for surface methanogenesis.

##### 4.4.2 Organic matter

The most important factor controlling benthic methanogenesis activity is probably the POC content of the sediment, as it determines the substrate availability and variety, and can thus relieve the competitive situation between methanogens and sulfate reducers (Holmer and Kristensen, 1994; Treude et al., 2009). Consequently, high methanogenesis rates may be expected along the Peruvian margin at sites with high organic carbon load. However, integrated methanogenesis rates did not correlate with sediment POC content (Fig. 6). While POC content was increasing from St. 1 (70 m) to St. 6 (253 m), followed by a decrease until St. 10 (1024 m), integrated methanogenesis showed rather a decreasing trend with increasing water depth. This deviation from expectations could be caused by organic matter quality, i.e., freshness. Numerous studies have shown that the quality of the organic matter is important for the rate and magnitude of microbial organic matter degradation (Westrich and Berner, 1984; Canfield, 1994; Amon et al., 2001; Middelburg, 1989).

Integrated methanogenesis and C/N ratios (indicating the freshness of organic matter) were negatively correlated along the Peruvian margin (Fig. 6), suggesting that fresh, labile organic matter is advantageous for surface methanogenesis. As methanogens consume mostly short, monomeric substrates, they depend on other microbial groups to break down large organic macromolecules (Zinder, 1993). Hence, labile organic matter offers an important supply of methanogenic substrates.

In agreement with this hypothesis, highest integrated methanogenesis rates were observed at St. 1 (70 m), which revealed the freshest organic matter (lowest C/N, Fig. 6) and the highest POC remineralization rates along the Peruvian margin (Dale et al., 2015). The degradation of organic matter within the water column was probably limited at St. 1 (70 m) due to anoxic conditions and high sedimentation rates (Dale et al., 2015); hence, labile organic matter accumulated at the seafloor, thereby increasing the benthic POC degradation and resulting in high substrate availability and variety for the methanogenic community.

Nevertheless, lowest methanogenesis rates were measured at St. 8 (407 m), which was neither the site of the highest C/N ratio, lowest POC content (Fig. 6), or the lowest POC mineralization (Dale et al., 2015). In this particular case, methanogenesis was most likely controlled by the sediment properties. Methanogenesis activity was undetectable below 10 cm b.s.f., which coincided with a very dense and sticky clay layer. The POC profile at St. 8 (407 m) revealed lower concentrations in the upper 5 cm b.s.f., followed by an increase with depth, suggesting that either the organic matter at this station was resistant to microbial attack (indicated

by the increase in C/N) or that microbes were not as frequent/active in the dense clay layer as at the surface. Similarly, sulfate reduction and microbial nitrogen fixation (Gier et al., 2015) showed very low activity at this site (Fig. 2).

## 5 Conclusions

The present study demonstrated that methanogenesis coincides with sulfate reduction in surface sediments (< 30 cm b.s.f.) along the Peruvian margin. The competition with sulfate reducers was partially relieved due to the high load of organic carbon allowing both groups to show concurrent activity through the utilization of non-competitive substrates by the methanogens.

The significance of surface methanogenesis was high on the shelf, where ratios between surface and deep methanogenesis were around 0.13 (this study) or even as high as  $\sim 10^5$  (compared to Arning et al., 2012), and decreased with increasing water depth. Accordingly, we assume that potential methane emissions into the water column, indicated by a higher methane concentration at the sediment surface compared to the bottom water, should be highest on the shelf, where surface methane production rates were highest. Our results further hint towards a partial consumption of methane before reaching the sediment–water interface, probably by anaerobic oxidation of methane (AOM). In this case, surface methanogenesis might act as important supplier of methane for AOM above the SMTZ, which has been largely overlooked previously.

We postulate that the dominant factor controlling surface methanogenesis is the availability of (primarily labile) organic matter. The high load of organic carbon and resulting high organic carbon mineralization rates secure the supply of methanogenic substrates, especially on the shelf, which mitigates the competition between sulfate reducers and methanogens. Anoxic conditions in the overlying water might be advantageous for the oxygen-sensitive process of methanogenesis, but does not appear to primarily control benthic rates, as they change within the anoxic zones.

Interestingly, organic matter made available by bioturbating infauna (e.g., fecal pellets or dead organisms) could be an important additional factor facilitating methanogenesis in surface sediments. As shown in this study, methanogenesis rates vary strongly in bioturbated sediments below the OMZ, sometimes exceeding all other observed methanogenic rates.

Future studies should seek to (1) identify methanogens and their metabolic capabilities in surface sediments, (2) determine the direct interaction between surface methanogenesis and AOM, and (3) evaluate the effect of organic matter hot spots on total benthic surface methanogenesis in organic-rich sediments.

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