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Methane excess production in oxygen-rich polar water and a model of cellular conditions for this paradox

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Abstract

Summer sea ice cover in the Arctic Ocean has undergone a reduction in the last decade exposing the sea surface to unforeseen environmental changes. Melting sea ice increases water stratification and induces nutrient limitation, which is also known to play a crucial role in methane formation in oxygenated surface water. We report on an excess of methane in the marginal ice zone in the western Fram Strait. Our study is based on measurements of oxygen, methane, DMSP, nitrate and phosphate concentrations as well as on phytoplankton composition and light transmission, conducted along the 79°N oceanographic transect, in the western part of the Fram Strait and in Northeast Water Polynya region off Greenland. Between the eastern Fram Strait, where Atlantic water enters from the south and the western Fram Strait, where Polar water enters from the north, different nutrient limitations occurred and consequently different bloom conditions were established. Ongoing sea ice melting enhances the environmental differences between both water masses and initiates regenerated production in the western Fram Strait. We show that in this region methane is in situ produced while DMSP (dimethylsulfoniopropionate) released from sea ice may serve as a precursor for the methane formation. The methane production occurs despite high oxygen concentrations in this water masses. As the metabolic activity (respiration) of unicellular organisms explains the

presence of anaerobic conditions in the cellular environment we present a theoretical model which explains the maintenance of anaerobic conditions for methane formation inside bacterial cells, despite enhanced oxygen concentrations in the environment.

1. Introduction

The Arctic Ocean is one of the regions in the world where climate change is most pronounced. Increased summer melting is considered to amplify biological production, due to the shift from an ice-covered to an open water Arctic Ocean (Arrigo et al., 2008). However, increasing water stratification during sea ice melting is likely to limit nutrient availability in near-surface water, which in turn hampers the enhancement of primary production (Sakshaug, 2003). A characteristic feature of the Arctic Ocean is the distinct post-bloom nutrient limitation found in the Atlantic-dominated and Pacific-dominated sectors. The former is nitrate and phosphate co-limited while the latter is mostly nitrate-limited, which results in an excess of phosphate (Yamamoto-Kawai et al., 2006). The role of nutrient limitation as a possible regulator of methane production in surface water has recently been investigated (Karl et al., 2008, Damm et al., 2010) while methane excess in ocean surface water relative to the atmospheric equilibrium has been studied for more than three decades (Scranton and Brewer, 1977). Different nutrient limitations can stimulate the growth of specific members of the bacterioplankton assemblage with consequences not only for the turnover of organic matter, biogeochemical cycling of carbon but also for producing climate relevant traces gases (Thingstad et al., 2008). Methanogenic archaea have been identified to have the ability to metabolize dimethylsulfoniopropionate (DMSP) and its degradation products by producing methane (Kiene et al., 1986; Oremland et al., 1989; van der Maarel and Hansen, 1997). However also bacteria may being methylotroph, using a series of methylated compounds, including methylated sulphur compounds such as (DMSP) and dimethylsulfide (DMS)

(Neufeld et al., 2008). This metabolism is referred as methylotrophic methanogenesis (Sowers and Ferry, 1983). DMSP is produced by marine phytoplankton and when metabolized, is a primary carbon source for heterotrophic bacteria (Kiene et al., 2000). DMSP is the precursor of dimethylsulfide (DMS) or methanethiol. DMS partly escapes to the atmosphere where it is the most important climate-cooling gas, counterbalancing the effect of greenhouse gases (Charlson et al., 1987). Methanethiol is a key reactive intermediate utilized as sulphur and carbon sources for biosynthesis or energy generation (Kiene et al., 2000). In anaerobic environments methanethiol act also as precursor for methane production (Tallant and Kryzcki, 1997). A switch in the utilization of phosphate and DMSP degradation products in nitrate-limited Pacific-derived water is also considered to produce methane in aerobic environments (Damm et al., 2010). Methane excess in surface water has also been detected under multi-year sea ice and in the marginal ice zone along the North-West Passage, i.e. the region from the southwest edge of Greenland through the Baffin Bay to the Beaufort Sea (Kitidis et al., 2010). Here we present data from Fram Strait where Atlantic water and Pacific-derived surface water bodies occur adjacent to each other. We show that ongoing sea ice melting has amplified the environmental differences between both water masses and we postulate that methane production occurs during regenerated production in Pacific-derived water despite an apparent oxygen excess. Methanogenesis in an aerobic environment is called the methane paradox as this process requires strictly anaerobic conditions. However, methane concentrations above the equilibrium concentration with the atmosphere are well known from the ventilated (i.e. oxic) open ocean surface layer (Reeburgh, 2007). Hence we determine the maximum oxygen concentration in seawater, which allows anaerobic processes to take place inside bacterial cells. Since this aspect is fundamentally important we provide a detailed model description to show and explain why and how it can potentially occur.

2. Study area

In the Fram Strait, the surface water (< 60 m) in general comprises two main water masses, which flow in opposite directions (Rudels et al., 2000). The warm (up to 4°C) and saline (up to 34.8) Atlantic water (AW) branch flows northward east of about 4°W (Fig.1). Further west, colder and less saline polar surface water (PSW) occupies the upper water column. In PSW, a portion is Pacific-derived water that varies inter-annually between more than 90% (Jones et al., 2003) to almost zero (Falck et al. 2005). In 2008, this portion had attained just over 60% (Dodd et al., 2012). The salinity of PSW was homogeneous at about 33 indicating unchanged conditions since winter convection, except for some near-surface warming and freshening by melt water (Fig. 2A). This distribution has been described previously for the end of the summer season (Budeus et al., 1997).

The recurrent Northeast Water Polynya (NEWP) is localized in the region of the PSW (Budeus and Schneider, 1995). Polynyas are less light-limited due to early opening of the ice cover compared to adjacent regions, and primary production starts earlier in the year. In the NEWP, nutrient-limited conditions occur at the end of July (Wallace et al., 1995, Kattner and Budeus, 1997). In the summer of 2008, ice fields drifting from the north partly covered the study area (Fig. 1). Hence stations in the middle of transect were located in partly ice-covered AW and PSW and the more eastern and western stations in ice-free AW and PSW, respectively.

3. Sampling and methods

In summer 2008, water sampling for measuring methane, oxygen, nutrients and DMSP was carried out in Fram Strait during the cruise ARK-XXIII/2 with RV “Polarstern”, roughly along the 79°N transect and spread on the Greenland sea shelf (Fig. 1). Further oceanographic and biological data were taken in the surface water to 200 m depths. The main sampling sites were along the hydrographic transect and in an opened ice lead on the Greenland shelf where

the sampling was repeated twice, first on July 23th (time 1) and one week later (time 2). Salinity, temperature, light transmission and oxygen were measured with a Seabird SBE 911+ CTD and C-Star Wetlabs transmissiometer. Oxygen was measured with the SBE 43 dissolved oxygen sensor SN 743 and sensor calibration was done on water samples using Winkler titration.

Water samples for estimating the abundance of dominant phytoplankton species were collected with a Niskin rosette sampling system and with an Apstein net (20 µm mesh size) towed through the upper 10 m of the water column. Samples were preserved in hexamine-buffered formalin (final concentration of ~1%) and dominant species or groups were counted with an inverted microscope. Nutrient analyses were performed on board with a nutrient analyzer (Evolution III, Alliance Instruments) according to standard methods. Methane concentrations were analyzed within a few hours after sampling. The dissolved gas was extracted from the water by vacuum-ultrasonic treatment and subsequently measured with a gas chromatograph (Chrompack 9003 (GC) with a flame ionization detector (FID). For gas chromatographic separation we used a packed column (Porapac Q 80/100 mesh). The GC oven was operated isothermally (60°C) and the FID was held at 250°C. Two sets of standard gas mixtures were used for calibration. The standard deviation of duplicate analyses was 5%. This high overall error is almost exclusively due to the gas extraction procedure and not to GC precision, which had an error of only 1%.

Total DMSP samples were collected directly from the Niskin sample bottles into 50 ml centrifuge tubes, containing 167 µl of 50% H_2SO_4, and stored at 4°C for later analysis. Dissolved DMSP samples were collected by the small volume drip filtration procedure recommended by Kiene and Slezak (2006). Briefly, immediately after sampling on the rosette about 50 ml of seawater was filled into a 47 mm filter tower with a Whatman GF/F glass fiber filter. From the water dripping through the filter only the first 3.5 ml of filtrate were collected directly into a storage tube containing 50 µl of 50% H_2SO_4. DMSP is stable for months in

129 acidic solution (Curran et al., 1999). In the home lab DMSP was analyzed as DMS after
130 alkaline cleavage. A subsample of the solution was pipetted into a 14 ml serum vial, treated
131 with 1 ml of 5 N NaOH and quickly sealed. The released DMS was purged into a cryotrap and
132 quantified with a gas chromatograph equipped with a Chromosil 330 column and a pulsed
133 flame photometric detector (PFPD). Helium was used as purge gas and carrier gas.

134

135 **4. Results and Discussion**

136 **4.1 Nutrient limitation and biological production**

137 In AW, nitrate and phosphate were abundant in the ice covered regions but depleted in open
138 waters, without changes in the Redfield ratio. In both ice-covered and ice-free PSW, nitrate
139 was undetectable in the near-surface layer (<20 m) and became limiting before phosphate
140 exhaustion (Fig. 2B and C). Hence, PSW was characterized by nitrate to phosphate ratios
141 lower than the Redfield ratio as also reported by Yamamoto-Kawai et al. (2006).
142 In addition to distinct nitrate availabilities, variations in oxygen saturation and light
143 transmissions along the E-W transect are obviously and point to bloom conditions which are
144 partly influenced by melting sea ice. As consequence, different blooms stages in ice free and
145 ice covered AW and PSW, respectively were eventually created (Fig. 2).
146 In the ice-free AW, the light transmission was reduced down to a depth of 60 m. This feature
147 is in accordance with a typical late bloom population which was observed in the non-stratified
148 water column east of 1°E (Fig. 2B and D). Besides the dominating prymnesiophyte
149 *Phaeocystis pouchetii*, many heterotrophic unicellular species were found belonging to
150 dinoflagellates and ciliates. Diatoms comprised a few *Thalassiosira* spp and very few
151 pennates. The occurrence of the two coccolithophores *Emiliania huxleyi* and *Coccolithus*
152 *pelagicus* was indicative of the minor ice influence. In comparison in the ice-free PSW an
153 impoverished phytoplankton community were found caused by the nitrate limitation and
154 reflected by the high light transmission (Fig. 2D, west of 10°W).

In the ice-covered regions both water masses clearly show a reduced light transmission up to 20 m depth. Sinking particles and ice algae released during brine drainage and ice melt may create this effect (Fig. 2B, Mundy et al., 2005). Under melting ice, chlorophyll concentrations are comparable in both water masses, ranging from nearly 0 to 2.6µg/L. Certainly the ice-covered AW was dominated by large *Phaeocystis pouchetii* colonies, which were partly covered with tiny pennate diatoms, whereas in the ice-covered PSW cold water ice-related algal communities were observed. In addition the different levels of oxygen saturation despite low solubility differences (by about 1.4%) were detected along the 79° transect which refers to deviations in the steady state between production and respiration in both ice-covered water masses (Fig. 2). Detected along the transect (Fig. 2) this observation is corroborated by the relation between chlorophyll and oxygen in a new opened lead in the NEWP region a region with long time ice-covered PSW (Figs. 1 and 3).

4.2 Sea ice melting in nitrate limited sea water - Biogeochemical consequences

The environmental differences between AW and PSW obviously create different under ice bloom conditions. While in the largely ice-covered AW new production occurred and favoured as nutrients are replete, a shift from new to regenerated production was evident in the nitrate-limited PSW (Fig. 2). The PSW on the East Greenland shelf is generally characterized by low initial nitrate concentrations (Kattner and Budéus, 1997). In summer, the surface waters are widely nitrate exhausted, and ammonium uptake becomes more important (Smith et al. 1997). The pronounced oxygen enhancement in the PSW combined with a highly variable nitrate to phosphate ratio revealed the importance of both new and regenerated production, probably dependent on the ice cover (Fig. 2E and 4). Thus, in PSW, where an excess of phosphate is available, ammonium could be an alternative nitrogen source to sustain

primary production. In the Fram Strait region, ammonium, released from multi-year Arctic sea ice, may additionally alleviate the nitrate limitation (Tovar-Sanchez et al., 2010). However, during regenerated production the ability of bacteria to compete with the phytoplankton community for inorganic nutrients and organic material is enhanced (Thingstad et al., 2008). Hence, melting sea ice in PSW may also affect the microbial food web. An important energy, carbon and sulphur source for bacterial biomass production is DMSP (Kiene et al., 2000). Both water masses differed clearly with regard to their DMSP concentrations. The high concentrations of DMSP in the ice-free AW were probably due to DMSP release by senescing *P. pouchetii* cells, which are known to be a major producer of DMSP in polar waters (Matrai and Vernet, 1997) (Fig. 2F). In comparison, low DMSP concentrations in the ice-free PSW may be due to an impoverished bloom of almost non DMSP producing diatoms, but perhaps also to an enhanced bacterial utilization of DMSP (Fig. 2F). The correlation between DMSP and oxygen saturation suggest a coupling of DMSP with the ongoing biological production in the AW (R^2=0.600; $p<0.001$). This correlation is however not found in the PSW (Fig. 5). The enhanced DMSP concentration in PSW is restricted to the upper 20 m and therefore likely induced by the DMSP release from melting sea ice (Fig. 2F, 5). The production of substantial amounts of DMSP by ice algae suggested by Levasseur et al. (1994) and Uzuka, (2003) corroborates this assumption. Furthermore DMSP released from sea ice is reported to be partially responsible for elevated DMSP concentrations in the water column at the ice edge (Trevena and Jones, 2006, Tison et al, 2010). A rapid microbial consumption of sea ice released DMSP (Galindo et al., 2014) suggest that sea ice released DMSP may serve as an additional carbon source for the microbial food web while finally the nutrient status in the water column impacts the pathway of its bacterial consumption.

4.2.1 Methane excess - a response to special environmental features?

The methane inventories were also clearly different in both water masses. In AW, methane concentrations tended to be in equilibrium or slightly under-saturated in relation to the atmospheric partial pressure (3 to 3.5 nM, depending on temperature and salinity). In the ice-free PSW a slight oversaturation was found, potentially generated by methane release from the seafloor in the NEWP region on the shallow shelf with water depths of about 100 m. In shallow polynya regions enhanced turbulence during convective mixing enhances sediment resuspension and eventually methane release from the seafloor (Damm et. al., 2007).

In the ice-covered PSW, however, a near-surface methane excess clearly rose above the slight oversaturation detected in ice-free PSW. It is striking that this methane surplus was found in the region where regenerated production occurred and where nitrate was clearly depleted (Fig. 2). This pattern is similar to that in the central Arctic Ocean where a change in the utilization of phosphate and methylated compounds is found to trigger the switch from no methane production to methane production in Atlantic and Pacific surface water, i.e. in nitrate/phosphate co-limited and nitrate-limited water (Damm et al., 2010).

Reduced turbulence in the presence of sea ice restricts the gas transfer (Rutgers van der Loeff et al., 2014). Hence the partially ice-covered water tends to reduce the escape of produced methane. Furthermore melting sea ice enhances the water stratification (Rabe et al., 2014). Indeed the PSW has a strong stratification in the upper water column induced by the long journey below melting sea ice (Fig 2A). Hence on one side, methane efflux and on the other side downward mixing is hampered in PSW. Both conditions finally induce that the methane excess created in sea ice-influenced water remains preserved during calm weather conditions in summer. Conspicuously is that the methane excess detected under multi-year-sea-ice and in the marginal ice zone along the North-West Passage (Kiditis et al., 2010) also occurs in a region which receives Pacific-derived water after its journey through the Arctic Ocean (Jones et al., 2003).

We therefore conclude that the development of a hotspot of methane production creates the excess as a rapid response during regenerated production when melting sea ice supplies DMSP, which may act as a potential precursor for methane formation. The microbial degradation of DMSP to methane was observed in a microcosm experiment carried out with seawater from the Fram Strait during this cruise (Damm et al., 2010). During the experiment *Archaea* abundance remained negligible and bacteria of the clades *Rhodobacter/Roseobacter* were dominant which are frequent in oligotrophic ocean surface waters and known for their highly diverse and flexible metabolism. A survey of available *Roseobacter* genomes by Moran et al. (2007) revealed that 50% of the genomes contained genes for DMSP demethylation.

4.3 Methane production in waters with oxygen excess - a paradox?

It is conspicuous that high oxygen concentrations in surface waters did not hamper methane production (Fig. 2E and G). As methanogenic activity is not favoured in an aerobic environment it was assumed that this process occurs in microenvironments which are sufficiently lacking in molecular oxygen (Cynar and Yayanos, 1992). The limiting conditions for the maintenance of a reduced micro-niche within oxidized marine sediments were first discussed by Jørgensen (1977). The question arises whether reducing conditions can exist, for example, within a *Roseobacter* cell, which would allow anaerobic processes inside the bacterial cell. To answer this question, we extended the model of Jørgensen (1977) by an additional compartment for the cell membrane and calculated the oxygen concentration profile in the interior of the cell as a function of the cell properties (cell size, rates of respiratory metabolism, membrane permeability for O_2) and the external O_2 concentration.

We describe the bacterial cell in terms of a sphere with a radius *b* covered by a thin membrane. The membrane is described by a homogeneous spherical shell of outer and inner radii *a* and *b* (Fig. 6A). Within the interior of the sphere there is an O_2 consumption of

constant intensity ρ. To determine the stationary concentration profile in the interior of the sphere we have to find the solution in the region $0 \le r \le b$ of

$$D\frac{1}{r^2}\frac{\mathrm{d}}{\mathrm{d}r}\left(r^2\frac{\mathrm{d}C(r)}{\mathrm{d}r}\right)-\rho(r)=0\,, \tag{1}$$

where D is the diffusivity of O_2 and $C(r)$ is the concentration of O_2 as a function of the radial distance r from the centre of the cell. Assuming a free floating cell, the diffusion coefficient (D) in the surrounding water is a constant and is given by the value in bulk seawater with a salinity of 33 at 0°C. Inside the cell, the salinity is probably slightly lower than in the surrounding seawater since a portion of the osmotic pressure in the cell is established by means of organic osmolytes. However, at 0°C a salinity change from 35 to about 30 (cellular interior) only has a minor impact on the diffusion coefficient for oxygen (S=30-35: D = 1.0580-1.0503 $\times 10^{-5}$ cm^2 s^{-1}, Ramsing and Gundersen, 1994). Our assumption therefore is that the diffusion coefficient of seawater ($D = D_w$) is the same for both the water in the cell and the surrounding water. Integrating and solving the equation for $\rho(r) = \rho$ and finite concentrations at $r = 0$ yields

$$C(r)=C_b-\frac{\rho}{6D_w}\left(b^2-r^2\right), \tag{2}$$

where $C_b = C(r = b)$ is the concentration in the sphere at the inner side of the membrane and D_w is the diffusion coefficient in water. To determine C_b we consider a stationary diffusion through the membrane of the thickness $h = a - b$ and with a permeability P for O_2. It is assumed that there is no O_2 consumption in the membrane region. Hence in the region $b \le r \le a$, equation (1) is integrated for $\rho(r) = 0$. With the total flux of O_2 through the membrane F (units: mol O_2 s^{-1}), the integration of equation (1) gives the following expression for C_b as a function of the concentration at the cell surface, $C_a = C(r = a)$,

$$C_b=C_a-\frac{F}{4\pi D_m}\frac{a-b}{ab}, \tag{3}$$

where D_m is the diffusion coefficient in the membrane and F equals the total O_2 consumption in the region $0 \le r \le b$, which is given by the respiration rate per cell, i.e. $F = \frac{4}{3}\pi b^3 \rho$. We consider the situation where the thickness of the membrane $h = a - b$ is small in proportion to the radius b. In this case $ab \approx b^2$, and equation (3) takes the form

$$C_b = C_a - \frac{F}{4\pi b^2}\frac{h}{D_m} = C_a - \frac{F}{4\pi b^2}\frac{1}{P} = C_a - \frac{\rho}{3}\frac{b}{P}. \quad (4)$$

To determine C_a we have to find the stationary concentration profile in the cell environment by solving equation (1) for $\rho(r) = 0$ in the region $r \ge a$. With $C(r \to \infty) = C_0$, integration of equation (1) yields

$$C(r) = C_0 - \frac{F}{4\pi D_w}\frac{1}{r} = C_0 - \frac{\rho}{3D_w}\frac{b^3}{r}, \quad (5)$$

where C_a follows for $r = a \approx b$. Replacing this value for C_a in equation (4) and the result for C_b in equation (2) gives

$$C(r) = C_0 - \frac{\rho b^2}{3D_w} - \frac{\rho b}{3P} - \frac{\rho}{6D_w}\left(b^2 - r^2\right), \quad (6)$$

which is the equation for the concentration profile in the interior of the cell. The strongest decline of $C(r)$ occurs across the membrane of permeability P (Fig. 6B). Inside the cell the diffusion of O_2 and respiration occur on two distinct time scales. Diffusion on the micrometer scale is, as a rule, much faster compared to slow metabolic processes. This means that within the cell significant concentration gradients of O_2 do not appear. According to equation (6) the concentration profile is described by a very flat parabola with the minimum of O_2 concentration at $r = 0$.

When $C(r)$ is zero for $r = 0$ we obtain the following equation for the maximum concentration in the environment, $C_{0,\max}$, which allows anaerobic processes to take place inside the cell:

$$C_{0,\max} = \frac{\rho b^2}{2D_w} + \frac{\rho b}{3P}. \quad (7)$$

For a salinity of 33 at 0°C, the diffusion coefficient of O_2 in water is $D_w = 1.05\times10^{-9}$ m^2 s^{-1} (Ramsing and Gundersen, 1994). The respiration per cell, 0.61 fmol O_2 h^{-1}, was measured in laboratory experiments with *Roseobacter* cultures grown in a chemostat at 22°C (Koblížek et

306 al., 2010). The simplest correction for the temperature of the bacterial respiration is the
307 temperature coefficient, Q_{10}, the factor by which a biological reaction changes with a
308 temperature increase of 10°C. The model of Rivkin and Legendre (2001) predicts a Q_{10} of
309 1.85 for bacterial respiration (Vázquez-Domínguez et al., 2007). The respiration rate
310 measured at 22°C is thus reduced by a factor of $1/Q_{10}^{2.2} = 0.26$ for an environmental
311 temperature of ~ 0°C. In the following a respiration rate per cell of F = 0.16 fmol O_2 h^{-1} at
312 0°C is therefore assumed. Using the cell volume of 0.53 μm^3 obtained in the laboratory
313 experiments with *Roseobacter* (Koblížek et al., 2010), it follows the radius of the sphere, b =
314 0.5 μm, and the constant intensity of O_2 consumption in the interior of the sphere, ρ = 0.084
315 mol m^{-3} s^{-1}. The permeability for gases of bacteria and microalgae has been determined in
316 very few investigations. The membrane permeability for $O_2(P)$ follows from the permeability
317 for CO_2 $\left(P_{CO_2}\right)$ by the relationship (Spalding and Portis, 1985)

318 $$P = P_{CO_2}\sqrt{\frac{\text{molecular weight of } CO_2}{\text{molecular weight of } O_2}}. \quad (8)$$

319 The inverse proportionality between P and the square root of molecular mass is assumed to
320 represent a useful approximation for gases that permeate the membrane via (passive)
321 diffusion. Using $P_{CO_2} = 3\times10^{-8}\,\text{m s}^{-1}$, as measured for *Synechococcus* UTEX 625 (Salon et al.,
322 1996), one obtains $P = 3.5\times10^{-8}\,\text{m s}^{-1}$. From equation (7) it now follows that the maximum O_2
323 concentration in the environment which allows anaerobic processes to take place inside the
324 bacterial cell, is $C_{0,max}$ = 400 μM. The latter corresponds to the O_2 concentrations observed in
325 nitrate-limited PSW in the region where the highest methane concentrations were observed
326 (Fig. 2E and G). Hence, in the PSW we had the situation where oxygen decreased almost to
327 zero in the interior of the bacterial cell, but was present in the membrane region and outside
328 the cell. The above calculations demonstrate that an oxygen excess in the surrounding
329 medium does not exclude the establishment of anaerobic conditions within the bacterial cell.

330 Altogether our model results suggest that oxygen excess and methane production are not
331 mutually exclusive.

332 ## 5. Summary and conclusions

333 A methane hotspot was detected in surface water of the western Fram Strait during summer
334 2008. We show that this methane excess is formed exclusively in Pacific-derived surface
335 water (PSW) where sea-ice melting occurred. It is not found in PSW without ice coverage
336 further west nor in Atlantic water (AW) further east. A conspicuous difference between both
337 water masses is the availability of nitrate which was clearly depleted in PSW. We show that
338 the methane excess is confined to a region where sea-ice is melting and postulate that DMSP
339 released from sea-ice act as the precursor of methane produced via methylotrophic
340 methanogenesis. Water stratification and reduced turbulence hampers the methane efflux
341 which finally induces the hot-spot in surface water. We prove by modelling that anaerobic
342 methanogenesis occurs inside a bacterial cell, despite high oxygen saturation levels in its
343 surrounding. These results support the observation that methane excess in stratified aerobic
344 seawater is coupled to an oligotrophic environment previously found in Pacific-derived water
345 in the central Arctic Ocean while a potential coupling of methanogenesis with DMSP
346 degradation processes requires further elucidation, especially the relationship between DMSP
347 turnover rates and in situ production of methane.

348
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479

480 **Captions**

481 Fig. 1: Map of the Fram Strait with ice coverage (AMSR-E data see Spreen et al., 2008). Ice
482 coverage is shown by colors from red to blue, (100%, 0%) meaning a closed ice cover and
483 open water, respectively. Black circles indicate stations localized in AW (Atlantic water) in
484 PSW (Polar surface water) and NEWP (Northeast Water Polynya)

Fig. 2: Profiles (black dots) along the transect from 15°W to 4°E (ice coverage ranges from 7°W to 0°); the transect crosses Atlantic water (east of 4.5°W) and Polar surface water (west of 4.5°W) diagrams show: potential density in sigma θ units (A), light transmission (%), (B), concentrations of nitrate, oxygen and phosphate (µmol/l) (C, D, E), concentrations of DMSP and methane (nM), (F and G).

Fig. 3: Oxygen saturation vs. chlorophyll *a* in Atlantic water (filled squares) oxygen is almost under saturated (up to 15%) and in Polar surface water (open squares) clearly oversaturated (by up to 11%) in relation to the atmospheric partial pressure. Dots, grey and black are from the new opened lead at time 1 and at time 2 (one week later), respectively. In AW and in the new opened lead clear different relationships are apparent while in PSW the data scatter between both ratios.

Fig.:4 Nitrate/phosphate ratios vs. oxygen concentration in Atlantic water (red squares/green triangles for ice-free and ice-covered stations) and Polar surface water (black/grey dots for ice-covered and ice-free stations). In the former, Redfield ratios are almost retained, while in the latter, nitrate limitation has induced increasing deviations from Redfield.

Fig. 5: Oxygen saturation vs. DMSP in Atlantic water (filled squares), in PSW (open squares) and in the new opened lead at time 1 and at time 2 (one week later), respectively. In PSW DMSP concentration higher than 20 nM are available in surface water (<20 m) and likely released from melting sea ice.

Fig. 6: (A) Model of oxygen distribution in a bacterial cell surrounded by oxic seawater; for explanation of symbols consult text.

(B) The oxygen concentration in the interior of the cell (*C(r)* of equation (6)) and in the cell membrane, where the concentration is described by the equation $C(r) = C_0 - \rho b^2/(3D_w) - \rho b^2/(3hP)(a/r - 1)$. Shown is the crucial role of the low membrane permeability (*P)* for the

maintenance of anaerobic conditions inside the cell. The dashed lines indicate oxygen concentration profiles for the n-fold increase (n=2, 4, 8, 16) in membrane permeability.