Methane Cycle in Terrestrial and Submarine Permafrost Deposits of the Laptev Sea Region

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Abstract

Permafrost environments within the Siberian Arctic are natural sources of the climate-relevant trace gas methane. In order to improve our understanding of present and future carbon dynamics in high latitudes, we studied the activity and biomass of the methanogenic communities in terrestrial and submarine permafrost deposits. For these investigations, permafrost cores of Holocene and Late Pleistocene age were drilled in the Laptev Sea region. A high CH_4 concentration was found in the upper 4 m of the Holocene deposits, which correlates well with the methanogenic activity and biomass. Even the incubation of core material at -3°C and -6°C showed a significant CH_4 production (range: 0.04–0.78 nmol CH_4 h^{-1} g^{-1}). The results indicated that the methane in permafrost deposits originated from modern methanogenesis by cold-adapted methanogenic archaea. Microbial-generated methane in permafrost sediments is, so far, an underestimated factor for future climate development.

Keywords: Laptev Sea; methane; methanogenesis; permafrost deposits; phospholipid biomarker; psychrophiles.

Introduction

The Arctic plays a key role in Earth's climate system, as global warming is predicted to be most pronounced at high latitudes and because one third of the global carbon pool is stored in ecosystems of the northern latitudes. Global warming will have important implications for the functional diversity of microbial communities in these systems. It is likely that temperature increases at high latitudes will stimulate microbial activity and carbon decomposition in Arctic environments, and accelerate climate change by increasing trace gas release (Melillo et al. 2002, Zimov et al. 2006). Currently, the functioning of microbial communities and their impact on changing environmental conditions are not adequately understood, and the potential methane release from frozen sediments is not adequately quantified.

Methane is chemically very reactive and more efficient in absorbing infrared radiation than carbon dioxide. Estimates of methane emissions from arctic and sub-arctic wetlands range between 10 and 39 Tg a^{-1}, or between 2.2 and 8.6% of global methane emissions (Bartlett & Harriss 1993, Cao et al. 1998). Methane, as a powerful greenhouse gas, contributes to about 20% of global warming (IPCC 2001).

In general, temperature is one of the most important variables regulating the activity of microorganisms. The growth potential, as well as the molecular, physiological and ecological aspects of microbial life at low temperatures, has been investigated in many studies (e.g., Gounot 1999, Wagner 2008). Certain key processes of the methane cycle are carried out exclusively by highly specialised microorganisms such as methanogenic archaea and methane oxidising bacteria. The microbial methane production (methanogenesis) in the active layer of permafrost is the terminal step during the anaerobic decomposition of organic matter, while the methane oxidation is the primary sink for methane in Arctic wetlands (Wagner et al. 2005).

However, there are only a few studies investigating the geochemistry and microbiology of permafrost deposits, which were mainly done in Siberia and Canada. Direct bacterial counts in the order of 10^7 to 10^8 were reported for permafrost deposits from Northeast Siberia (Rivkina et al. 1998). Shi and colleagues (1997) found viable bacteria in permafrost sediments up to 3 million years in age in the Kolyma-Indigirka lowlands. Most of the isolated bacteria showed mesophilic growth characteristics. In contrast, the minimum temperature for growth of permafrost bacteria was recently calculated to be -20°C (Rivkina et al. 2000). Furthermore, molecular life markers and low numbers of methanogens were found in the Mallik gas hydrate production research well (Colwell et al. 2005, Mangelsdorf et al. 2005). However, methanogenic activity could not be detected in the permafrost sediments using radiolabelled ^{14}C-substrates.

For the understanding and assessment of recent and future carbon dynamics in high latitudes, we have to answer the question: "What will happen to the carbon stored in permafrost, in the event of a climate change?" From this view point, we studied the methane concentration, the quantity and quality of organic matter, and the activity, biomass and diversity of methanogenic communities in permafrost deposits of the Laptev Sea region.

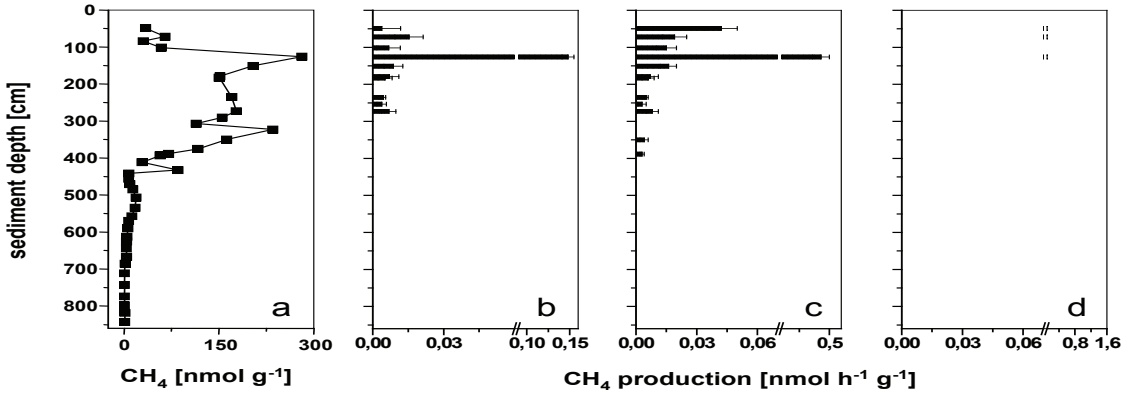

Figure 1. Vertical profiles of methane concentration (a), and methane production rates determined at 5°C without any additional substrate (b), with acetate (c) with hydrogen (d) as methanogenic substrates.

Study Sites

Within the scope of long-term studies on carbon dynamics in the Siberian Arctic, several expeditions were carried out by the Alfred Wegener Institute for Polar and Marine Research.

The Holocene permafrost core was drilled during the LENA 2001 expedition on the main study site Samoylov Island (72°22′N, 126°28′E, Pfeiffer & Grigoriev 2002). Samoylov, with the Russian-German Research Station, is located in the active part of the Lena Delta (Hubberten et al. 2006). The Lena Delta lies at the Laptev Sea coast between the Taimyr Peninsula and the New Siberian Islands. Continuous permafrost, which occurs throughout the investigation area, extends to depths of about 100–300 m (Yershov 1998), with active layer thicknesses between 30 and 60 cm depth.

The submarine permafrost cores of Late Pleistocene age were recovered in the framework of the COAST expedition from the western Laptev Sea along a transect running perpendicular to the coastline (Rachold et al. 2007). The Laptev Sea region is characterised by an arctic continental climate with low mean annual air temperature of about -15°C and low summer precipitation of <198 mm. Further details of the study sites were described previously in Wagner et al. (2003) and Rachold et al. (2007).

Drilling of Permafrost Deposits

The drilling of an 850 cm long core was carried out with a portable gasoline powered permafrost corer without using drilling fluid to avoid microbiological contamination of the permafrost samples. A mixing of the permafrost sediments was not observed due to the frozen state of the core material. The individual core segments, which were up to 50 cm in length, were placed immediately after removal from the corer into plastic bags and stored at about -8°C in the permafrost cellar of the Research Station Samoylov. After drilling of the core, the borehole temperature was monitored with a string of 9 thermistors. The cores were transported in a frozen state to Potsdam, Germany. During transport, the temperatures in the containers were monitored by micro data loggers. The storage temperature in the Potsdam laboratory was -22°C.

Core segments were split along their long axis into two halves under aseptic conditions with a diamond saw in an ice laboratory at -22°C. Afterwards, one half of the core was cleaned with a sterile knife for lithological and geocryological descriptions. Subsequently, one half was cut into segments of about 10–30 cm length according to the lithology and the geocryology. Small pieces (approx. 10 g) of each sub-sample were taken for analysing the methane concentration in the frozen sediments. The remaining material of each sub-sample was thawed at 4°C and homogenized under anoxic and sterile conditions for analysis of the sediment properties and the microbial activities and biomarkers. Sub-samples for the different analyses were placed into sterile plastic Nalgene boxes. Separated samples were used directly for the experiments (methane concentration, methane production rates, and biomarker analysis) or were freeze-dried for the organic carbon analyses. The second half of the core is kept as an archive in the ice core storage at the Alfred Wegener Institute.

Methanogenesis in Terrestrial Permafrost

Our results show significant amounts of methane in the first four meters of frozen sediments (up to 282 nmol CH_4 g^{-1} sediment, Late Holocene, 5000 yr BP until today) and only trace amounts of methane in the bottom section of the core (0.4–19 nmol CH_4 g^{-1} sediment; Middle Holocene, 9000–5000 yr BP; and Early Holocene, 11500–9000 yr BP; Fig.1a). Different amounts of methane in different aged permafrost deposits from northeastern Eurasia were reported by Rivkina & Gilichinsky (1996). They detected methane in modern (Holocene) and old permafrost deposits (Middle and Early Pleistocene, 1.8–0.78 mill. yr BP), but not in Late Pleistocene ice complexes (ice rich permafrost, 130000–11500 yr BP). They concluded from their findings that methane cannot diffuse through permafrost sections. If methane is unable to diffuse through permafrost from deeper deposits, it must either be entrapped during the deposition of the sediments or originate from recent methane production by methanogenic archaea (methanogenesis) in the frozen ground.

The analyses of methane production in selected sediment samples at 5°C, revealed activity only in permafrost layers with significant concentrations of methane (upper 4 m of the sediments; Fig. 1b). An important finding from the activity analyses is that no methane production was detectable in the bottom part of the permafrost section (>4 m) characterized only by traces of methane. This was also the case after addition of acetate or H_2/CO_2 as energy and carbon source (Fig. 1c, d). This indicates that the absence of methanogenesis does not depend on deficiency of methanogenic substrates in the Middle and Early Holocene deposits. Methane was only found in permafrost sediments with verifiable methane production activity.

The investigation of phospholipids as molecular biomarkers for *Bacteria* (PLFA) and *Archaea* (PLEL) shows a vertical profile with the same trend as the methane concentration. Specifically, significant amounts of phospholipids were determined in the upper Late Holocene deposits (<4 m sediment depth), which correlates (r = 0.632, P = 0.05) with the highest amount of methane (Fig. 2). In contrast, the biomarker concentrations in the Middle and Early Holocene permafrost sediments (>4 m sediment depth) drastically decreased to values below 10 nmol g^{-1} sediment, which corresponds with the detected traces of methane. Phospholipids are compounds of cell membranes that rapidly degraded after cell death (Harvey et al. 1986, White et al. 1979). They are regarded as appropriate biomarkers for viable microorganisms (e.g., Ringelberg et al. 1997, Zelles 1999). Therefore, the positive correlation of methane concentration with viable bacteria and archaea gives us the first strong evidence of recent methanogenesis under in situ conditions in permafrost deposits.

Although only a few psychrophilic strains of methanogenic archaea have been isolated, there are some indications of methanogenic activity in cold permafrost environments (Kotsyurbenko et al. 1993, Ganzert et al. 2006). However, the incubation of permafrost samples from 45–63 cm depth at sub-zero temperatures with acetate and hydrogen as methanogenic substrates, indicated a relatively high methane production rate under permafrost temperature conditions. At a temperature of -3°C, a significant increase in methane production was found, which rose linearly to headspace concentrations of about 1000 ppm (with acetate) and 2500 ppm (with hydrogen) during 300 h after the initiation of the experiment. At a temperature of -6°C, methanogenesis was lower; however, after a lag phase of about 300 h, a significant increase to 200 ppm (with acetate), and 500 ppm (with hydrogen) within 200 h, was observed. The calculated activity of methanogenic archaea with hydrogen reached values of 0.78 ± 0.31 nmol CH_4 h^{-1} g^{-1} and 0.14 nmol CH_4 h^{-1} g^{-1} at incubation temperatures of -3°C and -6°C, respectively. This was 2.5 and 3.5 times higher compared to the activity with acetate (0.31 ± 0.04 nmol CH_4 h^{-1} g^{-1} and 0.04 ± 0.01 nmol CH_4 h^{-1} g^{-1}) at the corresponding temperatures.

The quality of organic carbon is a limiting factor in the microbial metabolism process. Our results reveal a high organic carbon content (on average 2.4%) for the Holocene

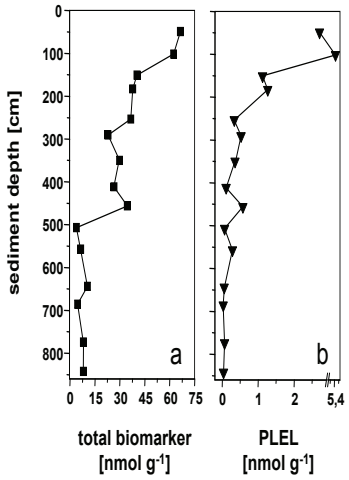

Figure 2. Vertical profiles of total lipid biomarkers (a) and phospholipid ether lipids (PLEL, b) within the Holocene permafrost core.

permafrost deposits (Table 1). However, the quantity of organic matter in permafrost ecosystems provides no information on the quality, which determines the availability of organic compounds as energy and carbon sources for microorganisms (Hogg 1993, Bergman et al. 2000). For this purpose, the humification index (HIX), which is a qualitative parameter, can give suitable information with regard to microbial metabolism. Wagner and colleagues (2005) demonstrated that the availability of organic carbon in permafrost soils decreased with increasing HIX. This is in agreement with the present study. It was shown for the permafrost sequence that the HIX increased continuously with depth. This indicates that the organic carbon is less available for microorganisms with depth because of the higher degree of humification. Consequently, at this point, we can summarize that the zone with significant concentrations of methane and activity of methanogenic microorganisms is characterized by the highest concentration of high quality organic carbon.

In contrast to the results of the soil-ecological variables (methane production activity, PLEL biomarker concentration, TOC, HIX), we do not achieve any hint for a possible entrapment process of methane during sedimentation, which was deduced from data of paleoclimate research carried out in the same study area (Andreev et al. 2004, Andreev & Klimanov 2005).

More than 20 percent of the terrestrial Arctic is characterized by ice rich permafrost (Zhang et al. 1999). Large areas, mainly dominated by continuous permafrost, exist in Siberia with thicknesses up to 900 m (Yershov 1998). The present study revealed that considerable parts of these cold habitats are recent sites of methane production, probably catalyzed by specific cold-adapted methanogenic archaea. This increasing reservoir of climate-relevant trace gases becomes of major importance against the background of global warming which could result from a thawing of

permafrost area up to 25% until 2100 (Anisimov et al. 1999) and subsequent disposal of the methane reservoirs into the atmosphere. Additionally, the results show that an increase of the permafrost temperature would lead to substantial rise in microbiologically-produced methane in the frozen ground. This would further strengthen the contribution of permafrost to the atmospheric methane budget.

Table 1. Borehole temperature, total organic carbon (TOC), and humification index (HIX, dimensionless) of the Holocene permafrost deposits from Samoylov Island.

Depth [cm]	T [°C]	TOC [%]	HIX
49	-1.9	4.82	3.71
72		2.50	5.74
84		3.64	5.33
102	-4	4.47	6.39
126		4.91	5.47
151		4.01	3.80
179		2.63	5.62
183	-7.4	3.42	6.64
235		n.d.	6.88
254		2.54	5.69
273		1.65	8.13
291		3.11	6.95
307	-9.4	0.87	0.68
323		1.88	6.08
350		2.11	6.83
375		2.49	8.01
389		n.d.	8.07
393	-12.5	n.d.	7.65
412		1.19	6.42
433		1.57	7.06
442		2.46	8.34
456		2.90	n.d.
471		3.00	7.65
485		2.54	8.10
507	-12.8	1.85	n.d.
534		2.27	8.25
557		2.49	8.70
570		2.65	7.80
590	-12.7	2.52	6.65
613		2.39	9.20
626		1.92	8.42
644		1.51	9.10
667		1.85	9.08
686		0.96	9.58
712		0.61	9.23
743		1.25	11.29
774		1.04	8.38
798	-11.5	1.69	9.11
819		1.97	9.46
843		2.56	8.42

n.d. = not detected

Methanogenesis in Submarine Permafrost

Coastal erosion and sea level rise created the shallow shelf of the Laptev Sea whose bottom is formed by the formerly terrestrial permafrost (Rachold et al. 2007). Flooding of the cold (-5 to -15°C) terrestrial permafrost with relatively warm (-0.5 to -2°C) saline sea water changed the system profoundly and resulted in a warming of the permafrost. Therefore, we consider submarine permafrost as a natural laboratory for studying the impact of environmental changes on permafrost habitats.

First results obtained from submarine permafrost deposits of the Laptev Sea shelf revealed methane concentrations of up to 284 nmol CH_4 g^{-1} sediment (Fig. 3a). Highest methane values were found in the layers with the highest amount of organic carbon (up to 9%). Extremely low $\delta^{13}CH_4$ values of -75 ‰ indicated active methanogenesis in this zone (Knoblauch, pers. com.). According to the studies of Rivkina & Gilichinsky (1996), who did not find any significant amounts of methane in Late Pleistocene permafrost sediments, it can be concluded that our findings in submarine permafrost are also a result of recent methanogenesis. This interpretation is supported by first data of DNA-based analyses of methanogenic communities in the sediments, which revealed a higher diversity and abundance of methanogens within the core segment with the highest amount of methane (Fig. 3b).

Conclusions

This work shows, for the first time, that methanogenic archaea do not only survive in permafrost habitats, but also can be metabolicly active under in situ conditions. Due to the sub-0°C experiments and the in situ temperatures of permafrost sediments, we can conclude that the methanogenic community is dominated by psychrotolerant or even psychrophilic microorganisms. Despite this adaptation to cold environments, we show that a slight increase of the temperature can lead to a substantial increase of methanogenic activity. In the event of degradation of terrestrial or submarine permafrost sediments, this would lead to an extensive expansion of the methane deposits with subsequent impacts on total methane emissions. A future in-depth characterization of the metabolism of these cold-adapted methanogens will reveal biotic and abiotic factors which influence the methane production activity of these organisms.

Methane of microbial origin in perennially frozen deposits probably represents an unconsidered source for the global methane budget. Methane release to the atmosphere from frozen ground is mediated by ongoing permafrost degradation through enhanced thermokarst formation and accelerated coastal erosion in the Arctic. Although the change in permafrost conditions by global warming is examined in the framework of several international projects (e.g., ACD: Arctic Coastal Dynamics, CALM: Circumpolar Active Layer Monitoring), these investigations should be linked more closely with microbiological process studies and biodiversity research. Microbial parameters important

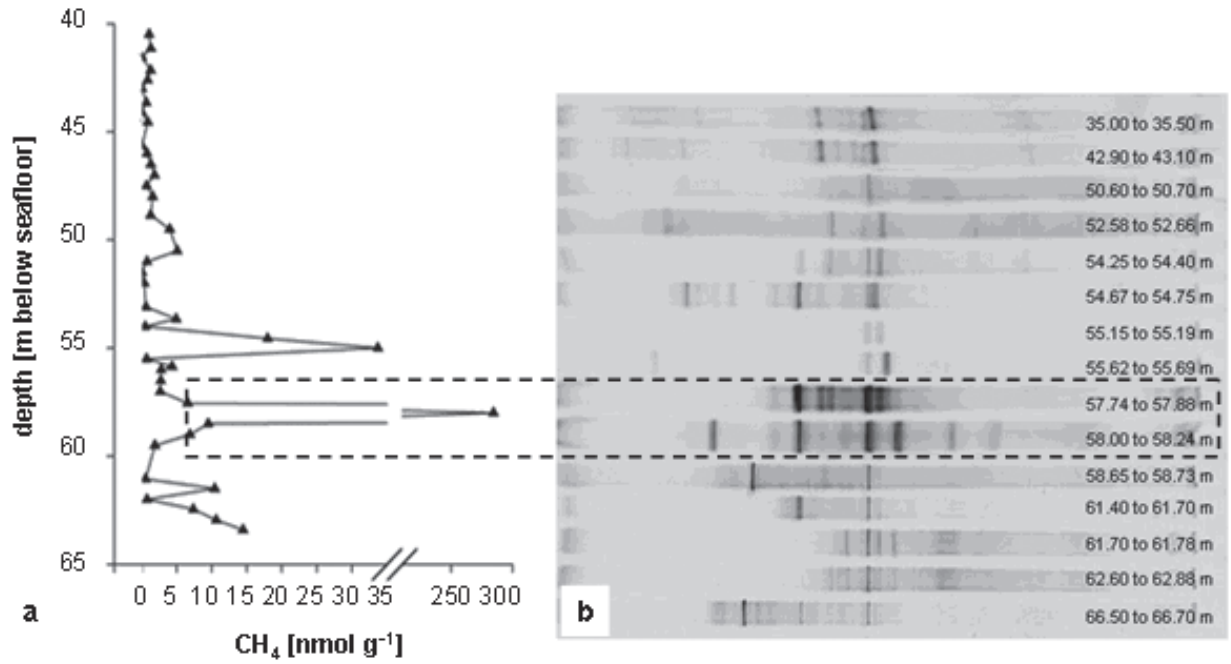

Figure 3. Vertical profiles of methane concentration (a), and DGGE fingerprinting of 16S rRNA genes (b) amplified from the submarine permafrost sediments (between 35.0 and 66.7 m depth).

for the assessment of the carbon turnover (e.g., cell numbers, activities, biodiversity and stability of microbial communities) should be analysed at observation areas in the Arctic, where long-term ongoing monitoring programs are undertaken. The evaluation of microbiological data and their correlation with climatic and geochemical results represents the basis for the understanding of the role of permafrost in the global system, in particular feedback mechanisms related to material fluxes and greenhouse gas emissions in the scope of a warming Earth.

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