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Methane fluxes in permafrost habitats of the Lena Delta: effects of microbial community structure and organic matter quality

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Summary

For the understanding and assessment of recent and future carbon dynamics of arctic permafrost soils the processes of CH_4 production and oxidation, the community structure and the quality of dissolved organic matter (DOM) were studied in two soils of a polygonal tundra. Activities of methanogens and methanotrophs differed significantly in their rates and distribution patterns among the two investigated profiles. Community structure analysis showed similarities between both soils for ester-linked phospholipid fatty acids (PLFAs) and differences in the fraction of unsaponifiable PLFAs and phospholipid ether lipids. Furthermore, a shift of the overall composition of the microbiota with depth at both sites was indicated by an increasing portion of iso- and anteiso-branched fatty acids related to the amount of straight-chain fatty acids. Although permafrost soils represent a large carbon pool, it was shown that the reduced quality of organic matter leads to a substrate limitation of the microbial metabolism. It can be concluded from our and previous findings first that microbial communities in the active layer of an Arctic polygon tundra are composed by members of all three domains of life, with a total biomass comparable to temperate soil ecosystems, and second that these microorganisms are well adapted to the extreme temperature gradient of their environment.

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Introduction

More than 14% of the global terrestrial carbon is accumulated in soils and sediments of Arctic permafrost environments (Post *et al*., 1982). Due to this carbon reservoir, tundra environments play a major role in the global carbon cycle, which is highlighted by current observed climate changes in the Arctic (IPCC, 2001) and by climate models that predict significant changes in temperature and precipitation in the Northern Hemisphere (Kattenberg *et al*., 1996; Smith *et al*., 2002). The atmospheric input of methane from tundra soils of high latitudes has been estimated between 17 and 42 Tg CH_4 $year^{-1}$ (Cao *et al*., 1996; Christensen *et al*., 1996), corresponding to about 25% of the methane release from natural sources (Fung *et al*., 1991). Particularly, the degradation of permafrost and the associated release of climate relevant trace gases, like CH_4 and CO_2 from intensified microbial turnover of organic carbon, represent a potential environmental hazard.

Permafrost, which particularly occurs in the Northern Hemisphere, covers more than 25% of the Earth's land surface (Zhang *et al*., 1999). These environments, which are under the influence of cryogenic processes, are characterized by patterned ground phenomena (Kessler and Werner, 2003). Low-centred ice-wedge polygons with a distinct microrelief (depressed centre, elevated rim) are one of the typical patterned grounds in tundra environments of northern Siberia. The microrelief affects the hydrological conditions as well as the organic matter contents and consequently the microbial processes.

The seasonal freezing and thawing leads to an extreme temperature regime in the upper active layer of permafrost. In spite of the extreme habitat conditions permafrost is colonized by high numbers of microorganisms including representatives of Archaea, Bacteria and Eukarya (Spirina and Fedorov-Davydov, 1998). In wet tundra soils methanogenesis is the terminal step during the anaerobic decomposition of organic matter, while the oxidation of methane by methanotrophic bacteria is the only sink for methane in these wetlands.

Generally, each habitat shows a characteristic composition of the microbial community, depending on the environmental conditions (Sundh *et al*., 1997; Gattinger *et al*., 2002a; Knief *et al*., 2003). Only few studies deal with

ecosystem-scale differences in methanogenic and methanotrophic communities concerning trace gas dynamics (Dunfield *et al*., 1993; Valentine *et al*., 1993; Steudler *et al*., 1996). Previous analysis of methane emission from polygonal tundra of the Lena Delta showed that the mean flux rate from the polygon depression was about 10 times higher compared with the CH_4 fluxes from the elevated polygon rim. These differences on the ecosystem level could be attributed to the different activity of the involved methanogenic and methanotrophic microflora as well as of the plant-mediated CH_4 transport (Wagner *et al*., 2003a; Kutzbach *et al*., 2004).

For the understanding and assessment of the recent and future carbon dynamic especially in sensitive high-latitude permafrost environments and the possible feedback to the atmospheric carbon budget the microbial processes have to be associated with the microbial community structure and functioning. The purpose of this study was to link methane production and oxidation activity with microbial community characteristics and the quality of dissolved organic matter in two different soils of a typical low-centred polygon. Special emphasis was given to the quantity and quality of water-extractable organic carbon (WEOC) and its function as a substrate for microorganisms. For community analysis of the polygon microbiota, we employed the polar lipid assay on samples from two soil profiles within the active layer of permafrost. The analyses included the determination of phospholipid fatty acids (PLFAs) and phospholipid ether lipids (PLELs) to enable detection of members of all three domains of the biosphere (Bacteria, Archaea and Eukarya).

Results

Soil characteristics

The microrelief formation of low-centred ice-wedge polygons leads to a small-scale variability in soil characteristics of the study site (Table 1).

The soils of the depressed polygon centre were dominated by *Typic Historthels*, whereas the prevalent soil type of the elevated polygon rim was classified as *Glacic Aquiturbel*. The thawing depth of both soils varied between 30 and 50 cm respectively. The peaty soil of the polygon centre was characterized by a water level near the soil surface and a soil texture of silty sand along with anaerobic accumulation of organic matter. Accordingly, large amounts of total organic carbon (TOC) and WEOC were determined, ranging between 36 and 183 mg g^{-1} and between 337 and 2239 μg g^{-1} dry weight (dw) respectively. The soils of the polygon rim were characterized by a soil texture of silty and loamy sand, pronounced cryoturbation properties, a distinctly lower water level causing oxic conditions in the top soil and a reduced organic matter accumulation. This is reflected by comparatively lower contents of TOC (21–33 mg g^{-1}) and WEOC (238–309 μg g^{-1}).

Analysis of the quality of WEOC revealed an increasing humification index (HIX) with increasing soil depth of the polygon centre. At the same time the bioavailable water-extractable organic carbon (BWEOC) content decreased (Fig. 1). Statistical analysis showed that both parameters were negatively correlated ($r = -0.84$) at the significance level $P < 0.01$.

Table 1. Selected soil properties of the depressed polygonal centre and of the elevated polygonal rim.

Horizon	Depth (cm)	CH_4 concentration (μmol g^{-1})	T (°C)	H_2O content (%)	pH	TOC (mg g^{-1})	N (%)	C/N	WEOC (μg g^{-1})	Sand (%)	Silt (%)	Clay (%)
Polygon centre (Typic Historthel)												
Oi1	0–5	0.15	7.5	72.2	n.d.	183	0.51	37.0	995	79.0	18.6	2.4
Oi2	5–10	13.19	5.8	67.4	7.9	138	0.43	33.1	2239	73.3	24.0	2.8
Ajj1	10–15	24.37	4.0	60.7	7.4	137	0.36	38.3	663	78.8	18.6	2.6
Ajj2	15–20	70.50	2.7	64.5	n.d.	93	0.23	41.5	349	76.6	15.4	7.9
Bg1	20–23	n.d.	1.2	60.3	n.d.	70	0.19	37.6	416	75.7	18.2	6.1
Bg2	23–30	163.24	0.4	55.0	n.d.	47	0.16	28.6	337	69.2	25.9	5.0
Bg2	30–35	328.87	n.d.	52.2	n.d.	36	0.15	24.6	440	67.7	27.0	5.3
Bg3	35–40	541.71	n.d.	52.6	n.d.	43	0.18	24.1	413	64.6	29.1	6.4
Bg3	40–45	n.d.	n.d.	47.9	n.d.	49	0.22	22.6	490	59.9	33.4	6.8
Polygon rim (Glacic Aquiturbel)												
Ajj	0–5	0.40	6.4	30.1	n.d.	21	0.12	17.8	n.d.	85.7	10.4	3.9
Bjjg1	5–12	0.29	5.0	27.5	n.d.	20	0.11	17.3	238	74.3	20.6	5.0
Bjjg2	12–20	35.26	4.0	26.2	7.9	24	0.14	17.1	309	68.0	25.8	6.3
Bjjg2	20–27	65.75	3.4	29.2	6.7	30	0.09	17.3	n.d.	63.7	30.3	6.0
Bjjg2	27–35	153.51	2.4	25.8	6.8	24	0.07	16.5	294	56.5	34.5	9.1
Bjjg3	35–42	224.71	1.7	26.1	n.d.	27	0.15	17.3	270	59.3	34.0	6.7
Bjjg3	42–49	478.74	1.0	28.4	n.d.	33	0.18	18.1	n.d.	43.7	43.8	12.5

Horizon nomenclature and soil classification according to Soil Survey Staff (1998); T, *in situ* temperature; TOC, total organic carbon; WEOC, water-extractable organic carbon; n.d., not detected.

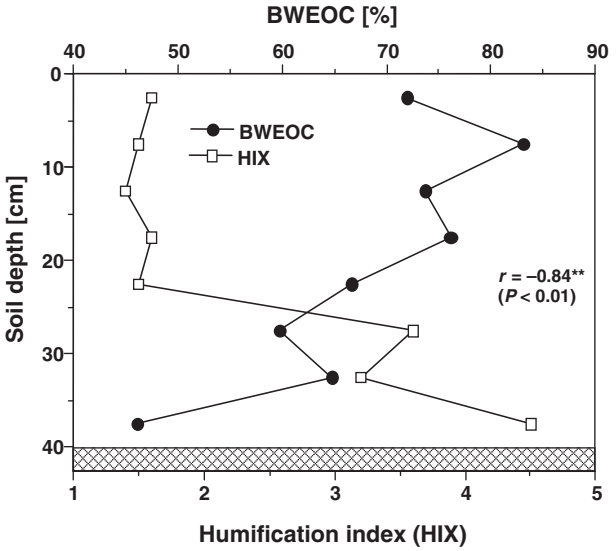

Fig. 1. Vertical profiles of bioavailable water-extractable organic carbon (BWEOC) and humification index (HIX, dimensionless) for the polygon centre. Bioavailable water-extractable organic carbon and HIX were negatively correlated at the significance level $P < 0.01$. Cross hatch indicates the frozen ground.

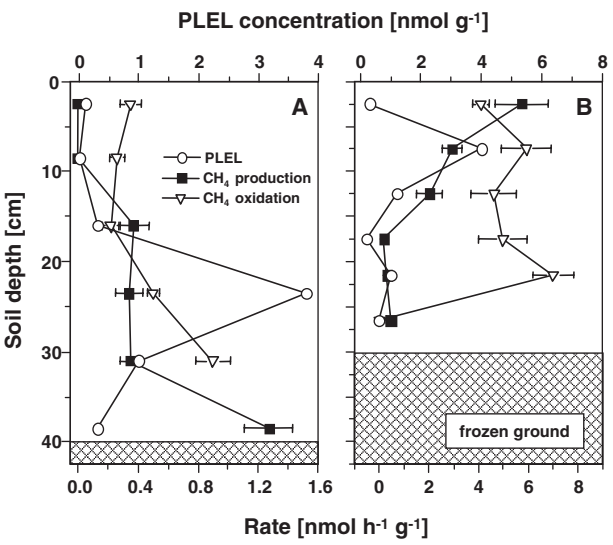

Fig. 2. Vertical profiles of CH_4 production and oxidation under *in situ* conditions as well as phospholipid ether lipid (PLEL) concentrations for a low-centred ice-wedge polygon determined in July/August 2001.
A. Polygon rim.
B. Polygon centre.

CH_4 production and oxidation

The microbial CH_4 production and oxidation activity in the soil of the polygonal rim (*Glacic Aquiturbel*) was much lower and showed another distribution than those appearing in the soil of the polygon centre (*Typic Historthel*). No CH_4 production was found in the upper soil layers (0–8 cm depth) of the elevated rim, which were dry and well aerated. The activity in the anoxic horizons (Bjjg) showed values from 0.3 to 1.3 nmol CH_4 h^{-1} g^{-1}. The highest CH_4 production was detected at the boundary to the frozen ground at an *in situ* temperature of about 1°C (Fig. 2A, Table 1). The oxidation capacities in the same profile varied between 0.2 and 0.9 nmol CH_4 h^{-1} g^{-1}. The highest oxidation rates were observed in the soil layer between 23 and 31 cm depth, where significant CH_4 production prevails. In contrast, the highest CH_4 production in the polygon centre was found in the top layer (5.7 nmol CH_4 h^{-1} g^{-1}), which decreased within the vertical profile and reached the lowest activity within the bottom zone with 0.2–0.3 nmol CH_4 h^{-1} g^{-1} (Fig. 2B). The CH_4 oxidation capacity was determined for the whole profile, which varied between 4.1 and 7.0 nmol CH_4 h^{-1} g^{-1}, except for the boundary to the frozen ground, where no CH_4 oxidation was detectable.

Methanogenic activity and concentration of archaeal PLELs followed the same trend in the polygon centre (Fig. 2B). However, no overall consistency between the CH_4 production under *in situ* conditions and PLEL concentration was found in the polygon rim (Fig. 2A). A better correlation between methanogenic activity and archaeal PLELs could be obtained, when substrates like acetate or hydrogen were added to the soil samples, as shown for the polygon centre (Fig. 3). In general, the potential CH_4 production rates were significantly higher compared with the activity under *in situ* conditions and reached values between 0.7 and 10.4 nmol CH_4 h^{-1} g^{-1} with acetate and 0.8–14.3 CH_4 h^{-1} g^{-1} with hydrogen.

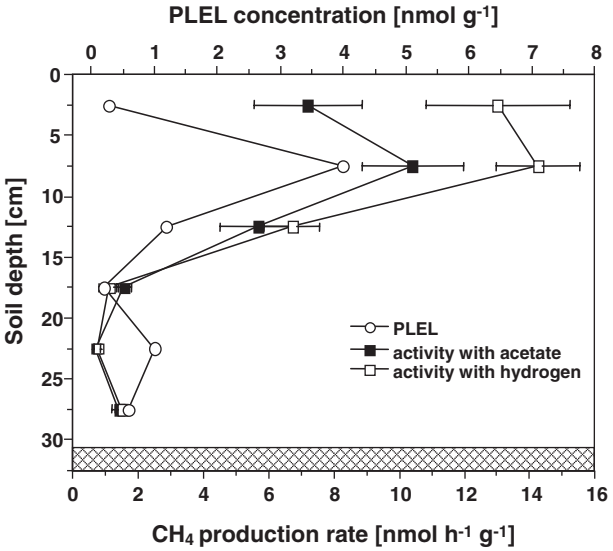

Fig. 3. Comparison of phospholipid ether lipid (PLEL) concentrations and potential CH_4 production after addition of acetate (20 mM) and hydrogen (v:v; 80:20) as methanogenic substrates for the polygon centre. Cross hatch indicates the frozen ground.

Phospholipid biomarker

In the polygon centre concentration of total phospholipid biomarker (PLFA + PLEL) ranged between 15.2 and 851.6 nmol g^{-1} dw (Table 2). The highest values were found in the depth between 5 and 10 cm, in contrast to the polygon rim, where highest concentration of total phospholipid biomarker was between 20 and 27 cm soil depths. In the polygon rim total phospholipid biomarker concentration varied between 20.5 and 105.5 nmol g^{-1} dw.

For comparison of microbial community composition, PLFA and PLEL data were subjected to principal component analysis (PCA; Fig. 4). The profiles of ester-linked PLFAs, which are the dominating fraction of phospholipids, were similar for the centre and rim samples (Fig. 4A). In contrast, the fraction of the unsaponifiable PLFAs and the PLEL fraction showed different profiles for rim and centre samples (Fig. 4B). The most important phospholipid biomarkers responsible for this separation were the anteiso-branched unsaponifiable PLFAs (UNSFA-ant), the straight-chain unsaponifiable PLFAs (UNSFA-nor) and the α-hydroxylated unsaponifiable PLFAs (UNOH-α) (Table 2). While the UNSFA-nor and the UNOH-α fractions were more abundant in the centre profile, the UNSFA-ant fraction dominated the unsponifiable PLFA fraction of the polygon rim.

The marker lipid for the type I methanotrophic family *Micrococcaceae*, *cis*-8-hexadecenoic acid (16:1Δcis8), was clearly detectable in both soils and showed its maximum concentration from 5 to 10 cm and from 20 to 27 cm depth in the polygon centre and rim respectively (Table 2). *Cis*-10-octadecenoic acid (18:1Δcis10), which is a marker lipid for the family *Methylocystaceae* (type II), was not detected in the polygon centre but at low concentrations in the upper two horizons (0–5 cm and 5–12 cm) of the polygon rim.

The fungal marker 18:2Δcis9,12 (Frostegard and Bååth, 1996) was detected in all investigated samples. The highest percentages of this PLFA were detected at 10–15 cm soil depth for the polygon centre (zone of maximum plant root growth) with 10.4% and in the first 5 cm of the polygon rim with 9.2%.

A shift of the overall composition of the microbiota with depth at both sites was indicated by an increasing portion of iso- and anteiso-branched fatty acids related to the amount of straight-chain fatty acids. The ratio of 4.7 of straight-chain to iso- and anteiso-branched fatty acids at 0–5 cm depths for the centre sample and of 7.2 for the rim sample decreased to a ratio of 2.3 and 2.1, respectively, at the bottom of both soil profiles (Table 2).

Phospholipid ether lipid-derived isoprenoids (biomarker for archaea) were detected in all samples and were highest in the soil depths 5–10 cm (4.0 nmol g^{-1}) for the polygon centre and 20–27 cm (3.8 nmol g^{-1}) for the polygon rim (Table 2). Most of the samples contained only the two ubiquitous archaeal markers phytane and biphytane (i20:0 and i40:0). Only in the soil depth 5–10 cm from the polygon centre the side-chain i20:1 was found (data not shown), indicating the presence of acetoclastic methanogens (Gattinger *et al.*, 2002a). The PLEL biomarker in relation to the total phospholipid concentration increased in both vertical profiles with increasing soil depth and reached a maximum of 3.4% near the bottom layer of the centre and 3.6% in 20–27 cm soil depth of the rim (Table 2).

Discussion

Permafrost, a common phenomenon in the Siberian Arctic, is controlled by climatic factors and characterized by extreme terrain conditions and landforms (Wagner *et al.*, 2001). The seasonal unfrozen part of permafrost (active layer, approximately 0.5 m thickness at the study site) is subjected to freezing and thawing cycles during the year with an extreme surface temperature from about 25°C to −45°C. In geological timescales cryogenic processes lead

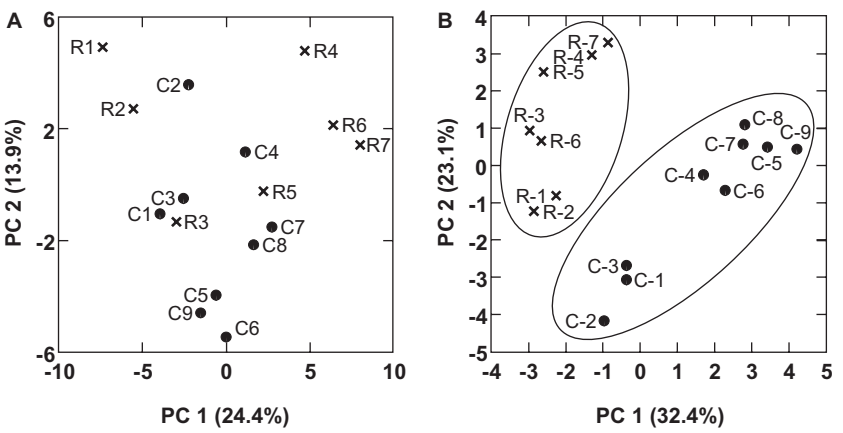

Fig. 4. Principal component (PC) diagram of ester-linked phospholipid fatty acids (A), as well as unsaponifiable phospholipids and phosphoether lipids (B). The numerals 1–9 designate the different sampling depths (R, polygon rim; C, polygon centre).

Table 2. Concentrations of selected phospholipid biomarker (PLFA and PLEL).

			Ester-linked PLFA				Non-ester-linked PLFA				PLEL	
Sample ID	Depth (cm)	PLFA + PLEL (nmol g^{-1})	Total (nmol g^{-1})	16:1Δcis8 (nmol g^{-1})	18:1Δcis10 (nmol g^{-1})	Straight/ iso + anteiso	Total (nmol g^{-1})	UNSFA-nor (nmol g^{-1})	UNSFA-ant (nmol g^{-1})	UNOH-α (nmol g^{-1})	Total (nmol g^{-1})	(%)
Polygon centre (Typic Historthel)												
C1	0–5	26.3	24.5	0.1	0.0	4.7	1.5	1.3	0.0	0.0	0.3	1.0
C2	5–10	851.6	830.6	4.4	0.0	4.6	17.0	6.7	0.5	3.4	4.0	0.5
C3	10–15	250.3	231.6	1.0	0.0	4.3	17.5	9.3	0.6	1.9	1.2	0.5
C4	15–20	83.5	65.9	0.3	0.0	3.4	17.5	3.0	0.8	6.3	0.2	0.2
C5	20–23	39.0	25.8	0.1	0.0	3.1	12.2	5.4	0.6	2.2	1.0	2.6
C6	23–30	23.4	16.9	0.1	0.0	2.6	5.9	3.1	0.0	1.5	0.6	2.4
C7	30–35	54.0	41.3	0.2	0.0	2.7	11.3	4.7	0.2	1.7	1.4	2.6
C8	35–40	53.0	38.7	0.1	0.0	2.5	12.4	2.7	0.5	5.0	1.8	3.4
C9	40–45	15.2	8.0	0.0	0.0	2.3	6.8	3.9	0.2	0.7	0.4	2.7
Polygon rim (Glacic Aquiturbel)												
R1	0–5	40.7	33.4	0.2	0.2	7.2	7.2	0.1	2.2	0.3	0.1	0.1
R2	5–12	20.5	17.6	0.1	0.1	5.7	2.9	0.0	1.7	0.1	0.03	0.2
R3	12–20	24.7	18.7	0.1	0.0	3.7	5.8	0.1	2.5	0.4	0.3	1.1
R4	20–27	105.5	82.4	0.6	0.0	2.6	19.3	0.0	3.8	1.7	3.8	3.6
R5	27–35	42.3	32.3	0.2	0.0	2.3	9.0	0.1	3.5	0.5	1.0	2.4
R6	35–42	60.9	50.4	0.5	0.0	2.5	10.3	0.5	3.5	0.6	0.3	0.4
R7	42–49	53.6	38.2	0.3	0.0	2.1	13.7	0.0	2.9	1.1	1.7	3.1

Subgroups of the non-ester-linked phospholipid fatty acids (PLFAs) were the unsubstituted (UNSFA) and hydroxy-substituted fatty acids (UNOH). Subgroups of UNSFA were named according to their functional groups: '-ant' (anteiso-branching), '-nor' (normal straight chain), '-uns' (unsaturations). UNOH subgroups were named according to the position of the hydroxy group in the fatty acid molecule ('α' or 'mid' position). 'ant/iso' describes the molar ratio of anteiso- to iso-branched ester-linked PLFAs and 'unsat/sat' the molar ratio of unsaturated to saturated ester-linked PLFAs.

to the formation of patterned grounds like the low-centred ice-wedge polygons of the investigation area in the Lena Delta. During the summer period soils within these polygons are also showing a large temperature gradient along their depths profiles, which is one of the main environmental factors influencing the microbial community in permafrost soils. The presented results revealed differences between the microrelief elements of the investigated polygon (elevated rim and depressed centre) in CH_4 fluxes, the microbial community structure and soil characteristics on the ecosystem scale (centimetres to metres).

Activities of methanogens and methanotrophs differed significantly in their rates and distribution patterns among the two investigated permafrost profiles. While the CH_4 production and oxidation in the polygon rim showed the typical activity patterns as known from other hydromorphic soils (Krumholz *et al*., 1995), which means no or less activity in the dry and oxic upper horizons and increasing rates in the anoxic bottom layers, this is not the case in the polygon centre. Here the highest CH_4 production occurred in the upper soil horizons with a redox potential of about −50 mV, as shown in former studies at the same investigation site (Fiedler *et al*., 2004). This is not the appropriate redox regime for CH_4 production. However, it was shown that a complex community composed by aerobic and facultative anaerobic microorganisms together with a certain soil matrix enables CH_4 production under oxic conditions (Wagner *et al*., 1999). Integrated analyses of phospholipid biomarker revealed soil layers with a good relationship between the concentrations of archaeal PLELs as well as total phospholipid biomarker (indicator for microbial biomass) and CH_4 production under *in situ* conditions but there were other zones in the profiles without any correlation between both parameters (Fig. 2). Nevertheless, a stronger relationship was observed when archaeal PLEL concentration was compared with potential CH_4 production, as shown for the polygon centre. This finding indicates a substrate limitation for methanogenesis although organic carbon is highly accumulated in permafrost soils. Subsequent organic matter analyses revealed a decrease of BWEOC along with an increasing HIX with increasing soil depth. Accordingly, WEOC showed the highest values in the soil horizons of highest methanogenesis and archaeal PLEL concentration (Tables 1 and 2).

CH_4 oxidation capacities followed the curve of CH_4 production in the polygon rim, whereas in the centre CH_4 oxidation capacities were relatively high within the whole profile with exception of the bottom layer. The signature PLFA 18:1Δcis10 for the two methanotrophic genera *Methylosinus* and *Methylocystis* of the α-*Proteobacteria* was detected only in the polygon rim at 0–12 cm soil depth. In contrast, the PLFA 16:1Δcis8 indicative for the genera *Methylomonas*, *Methylomicrobium*, *Methylosarcina* and *Methylosphaera* (Bowman *et al*., 1993; 1997; Wise *et al*., 2001) was in accordance with the CH_4 oxidation capacities in both soils. *In situ* labelling of corresponding samples with ^{13}C-enriched CH_4 supported our findings and revealed a significantly higher incorporation of labelled carbon into PLFAs belonging to type I methanotrophs (U. Zimmermann and A. Gattinger, unpublished results), all of them belonging to the group of γ-*Proteobacteria.* Furthermore, cell numbers of γ-*Proteobacteria* determined by FISH were closely correlated with the CH_4 oxidation profile in the polygon centre (Kobabe *et al*., 2004). The activity of methanotrophic bacteria in the bottom layer of permafrost soils can be explained by high substrate affinity of type I methanotrophs (Hanson and Hanson, 1996) and by the plant-mediated transport of O_2 into the rhizosphere (Kutzbach *et al*., 2004).

The high variability of environmental conditions within the polygon is reflected by the large differences in CH_4 emission from the different areas of this microrelief obtained by long-term studies since 1998. For example, in 1999 the mean flux rate of the polygon centre measured from the end of May to the beginning of September was 53.2 mg CH_4 m^{-2} day^{-1}, while the dryer rim part showed a mean value of 4.7 mg CH_4 m^{-2} day^{-1} (Wagner *et al*., 2003a). The reason for this large spatial variability in CH_4 emission can be explained by the activity patterns of methanogens and methanotrophs, which are interacting with complex microbial communities. These showed differences in biomasses and structures between polygon rim and centre as revealed in the presented study by detailed phospholipid profiling. UNSFA-nor, UNSFA-ant and UNOH-α were identified as three of most responsible PLFAs for separation into the two major groups 'rim samples' and 'centre samples' according to PCA. UNSFA-nor occur, for example, in high concentrations in fermentative bacteria such as *Clostridia* (Gattinger *et al*., 2002b) and in moderate concentrations in methanogens isolated from the investigation site as shown by ^{13}C-acetate labelling experiments (D. Wagner and A. Gattinger, unpublished results). UNSFA-ant were found in high concentrations in *Cytophaga* sp., whereas UNOH-α were determined in *Alcaligenes* sp. and *Flavobacterium* sp. (Zelles, 1999).

Although permafrost environments are characterized by extreme temperature conditions, the CH_4 emissions from these ecosystems (219–329 kg C ha^{-1} a^{-1}; calculated from Wagner *et al*., 2003a) are in the same range compared with boreal (190–480 kg C ha^{-1} a^{-1}; Martikainen *et al*., 1995) or temperate fens (11–293 kg C ha^{-1} a^{-1}; Augustin *et al*., 1996). The maximal values for microbial biomass (total phospholipid biomarker concentrations) of 105.5 and 851.6 nmol g^{-1} dw for the polygon rim and centre, respectively, are significantly higher than in arable soils (35.2–59.4 nmol g^{-1}, Zelles, 1999; Gattinger *et al*.,

2002a), rice paddies (44.7–90.9 nmol g^{-1} dw, Bai *et al*., 2000) and boreal Swedish peatlands (0.2–7.0 nmol g^{-1} wet peat, Sundh *et al*., 1997). The maximum value of 851.6 nmol g^{-1} determined in the polygon centre is within the range of a landfill cover soil studied by Börjesson and colleagues (2004).

The function of the cell membranes especially at low temperatures is highly dependent on the fluidity of the membrane (Ratledge and Wilkinson, 1988). There are several mechanisms known for the adaptation of the membrane fluidity under cold conditions. These are increases of the proportion of anteiso-branched to iso-branched fatty acids or of unsaturated to saturated fatty acids, because anteiso-branched fatty acids and unsaturated fatty acids have significantly lower melting points than their iso-branched and saturated analogues (Kaneda, 1991). At the study site the mean anteiso/iso ratio was 1.2 for the polygon centre and the rim respectively. Furthermore, the mean unsaturated/saturated ratio was 1.4 for the polygon centre and 1.6 for the rim. These ratios were significantly higher than those from ester-linked PLFAs determined for temperate soil microbial communities, which showed anteiso/iso ratios from 0.3 to 0.9 and unsaturated/saturated ratios from 0.1 to 0.5 (calculated from data of Zelles and Bai, 1994).

These findings along with the determined CH_4 production and oxidation activities, which were independent of the temperature gradient in the active layer, show an adaptation of the microbial community to the low permafrost temperatures. This is also in accordance with the determination of high cell numbers of 1.2×10^8 cells per gram of soil in the boundary layer to the permafrost in the polygon centre, which had a relatively constant temperature regime of 1°C (Kobabe *et al*., 2004). In the same study only a minor part of the Eubacteria (EUB)-positive staining cells could be identified in the bottom layers by the common FISH probes, which shows on the one hand that a large part of species did not fit into the phylogenetic groups detected with used FISH probes and on the other hand it indicates probably a large number of unknown organisms in permafrost soils.

Although the fungal PLFA 18:2Δcis9,12 occurs also in a few bacterial species (see Zelles, 1997), one can assume the presence of fungi (Domain Eukarya) for the investigated soils. Hence it can be concluded that microbial communities in the active layer of an Arctic polygon tundra are composed by members of all three domains of life (Archaea, Bacteria and Eukarya) yielding a total biomass comparable to temperate soil ecosystems (Zelles, 1999; Bai *et al*., 2000; Gattinger *et al*., 2002a). At the same time the composition of the microbial communities and the activities of methanogens and methanotrophs are mainly influenced by the microrelief formed by cryogenic processes, which leads to different microenvironments. The permafrost environment forces the adaptation of the microbial communities to low temperature conditions with a significant proportion of unknown species. Although the total amount of organic carbon in the depressed centre is significantly higher compared with the elevated rim, the methanogenesis is substrate limited because of a decreasing bioavailability of organic carbon within the soil profile. This is an important finding for modelling and calculating trace gas fluxes from permafrost environments, because the known models consider only the total carbon amount. Further integrative analyses are planed for detailed functioning analysis and forecasting of the development of permafrost environments under changing climate conditions.

Experimental procedure

Study site

Within the framework of the Russian–German cooperation 'System Laptev Sea 2000' an expedition to Northern Siberia was carried out in summer 2001 (Pfeiffer and Grigoriev, 2002). The study site Samoylov Island (N 72°22, E 126°28) lies within the active and youngest part (about 8500 years) of the Lena Delta, which is one of the largest deltas in the world with an area of 32 000 km^{-2} (Are and Reimnitz, 2000). It is located at the Laptev Sea coast between the Taimyr Peninsula and the New Siberian Islands in the zone of continuous permafrost. The Lena Delta is characterized by an arctic continental climate with low mean annual air temperature of −14.7°C (T_{min} = −48°C, T_{max} = 18°C) and a low mean annual precipitation of 190 mm.

Soil and vegetation characteristics vary in rapid succession at the investigation site due to the patterned ground of low-centred ice-wedge polygons, which were formed by the annual freezing–thawing cycles. Accordingly, one investigation profile was located in the depressed polygonal centre and the other one at the elevated polygonal rim. The distance between the two investigated soils was about 10 m. The soil surface of the polygon depression was about 0.5 m below the surface of the elevated rim part. Further details of the study site were described previously by Wagner and colleagues (2003a).

Soil properties

Vertical profiles of soil CH_4 concentrations were obtained from both the elevated rim and the depression centre of the polygon by extracting CH_4 from soil pore water by injection of 5 ml of water into saturated NaCl solution, shaking the solution and subsequently analysing the CH_4 headspace concentration with gas chromatography. Soil temperature measurements (Greisinger GTH 100/2 equipped with Ni-Cr-Ni temperature sensor) were carried out during the experiments of CH_4 production and oxidation under *in situ* conditions (5 cm increments from 0 to 40 cm soil depth).

The investigated soils were classified according to the US Soil Taxonomy (Soil Survey Staff, 1998). Soil properties were described during sampling (horizontal stepwise) according to

Schoeneberger and colleagues (2002) and soil chemical and physical analyses were performed according to Schlichting and colleagues (1995). Samples were filled into 250 ml Nalgene boxes and transported in frozen conditions to Germany. Further details of the sample procedure were described elsewhere (Kobabe *et al*., 2004).

CH_4 production and oxidation

The CH_4 production and oxidation capacity of the soils were analysed in summer (July to August) 2001. The CH_4 production was studied considering the *in situ* soil temperature gradient and different methanogenic substrates like H_2 and acetate (CH_4 production potential). Fresh soil material (20 g) from different soil horizons was weighed into 100 ml glass jars and closed with a screw cap containing a septum. The samples were evacuated and flushed with ultrapure N_2 (*in situ* CH_4 production). In the case of analysing the CH_4 production potential fresh soil material was supplied with 6 ml of acetate solution (10 mM) or with sterile and anoxic tap water in combination with H_2/CO_2 (80:20 v/v, pressurized 150 kPa) as methanogenic substrates. The CH_4 oxidation capacity was studied considering the *in situ* CH_4 concentration and the natural soil temperature gradient. Fresh soil material (5 g, well homogenized) from different soil horizons was weighed into 50 ml glass jars and closed with a screw cap with septum. The samples were supplied with about 2000 p.p.m. CH_4 (corresponding to about 800 μmol CH_4 l^{-1} pore water) in synthetic air. The prepared soil samples were restored for incubation in the same layers of the soil profile from which the samples had been taken. Three replicates were used for each layer. Gas samples were taken every 24 h for CH_4 production and every 12 h for CH_4 oxidation out of the jars headspace with a gastight syringe. CH_4 production and oxidation rates were calculated from the linear increase or decrease in CH_4 concentration analysed by gas chromatography.

CH_4 analysis

CH_4 concentrations were determined with a gas chromatograph (Chrompack GC 9003) in the field laboratory. The instrument was equipped with a Poraplot Q (100/120 mesh, 20 m) capillary column and a flame ionization detector (FID). Details of CH_4 analysis were described previously (Wagner *et al*., 2003b).

Total and water-extractable organic carbon

Total organic carbon was analysed with an element analysator (Elementar Vario EL) using dried and homogenized soil samples. Before analysis the samples were treated with HCl (10%) at 80°C for carbonate removal.

The WEOC was quantified with a batch extraction method. Frozen soil samples were extracted with a 10 mM $CaCl_2$ solution using a soil:extractant ratio (w/w) of 1:10 and shaking for 10 min in an overhead shaker. Subsequently, the suspensions were centrifuged for 15 min (4000 r.p.m.) and the supernatants were filtered through 0.45 μm polycarbonate filters (Millipore, Eschborn, Germany). Filtered solutions were quantified for dissolved organic carbon using catalytic high temperature combustion (680°C) with a Shimadzu® TOC 5050A analyser. Non-organic carbon was removed by acidification and purging the samples with pure O_2 for 2 min before measurement. The WEOC concentrations were referred to weighted soil mass (dry matter) and expressed as μg C g^{-1} dry matter.

Water-extractable organic carbon quality was quantified using optical measurements (UV absorption and fluorescence emission intensity). The fluorescence emission intensity was measured between 300 and 480 nm with an excitation wavelength of 254 nm (Cary Eclipse F-4500, Varian®). Before measurement, soil extracts were adjusted to pH 2, due to the influence on soil pH on fluorescence of organic molecules (Zsolnay *et al*., 1999). Based on the fact that highly substituted aromatic structures and condensed unsaturated systems fluoresce in the longer wavelength and fresh, non-humified organic matter fluoresce in the shorter wavelength (Senesi *et al*., 1989), the HIX was calculated by dividing the upper quartile (435–480 nm) of the whole spectrum through the lower quartile (300–345 nm). The higher the (dimensionless) HIX, the more dissolved organic carbon in the samples is humified (Zsolnay, 2003).

Bioavailable water-extractable organic carbon was quantified mixing 5 ml of WEOC extract with 2 ml of nutrient solution (1 ml of NH_4NO_3 + 1 ml of K_2HPO_4, each at a concentration of 1 g l^{-1}) in Teflon vessels. After adding 30 μl of soil inherent inoculum (reference culture, obtained from the supernatant of a suspension of 50 g of pooled sample from rim and centre soil – each horizon in equal amounts – with 50 ml of drinking water) the closed vessels were incubated in the dark at room temperature for 7 days. Bioavailable water-extractable organic carbon was calculated by subtraction of $WEOC_{day\ 0}$ – $WEOC_{day\ 7}$ and expressed in percentage of the initial $WEOC_{day\ 0}$. For further descriptions as well as for advantages and disadvantages of this method see Marschner and Kalbitz (2003)

Lipid extraction of soil samples

Lipids were extracted from a fresh soil sample equivalent to a dry weight of 50 g, according to the Bligh-Dyer method as described elsewhere (Zelles and Bai, 1993). The resulting lipid material was fractionated into neutral lipids, glycolipids and phospholipids on a silica-bonded phase column (SPE-SI; Bond Elute, Analytical Chem International, CA, USA) by elution with chloroform, acetone and methanol respectively.

Determination of PLFAs and PLELs

Both assays are based on the determination of phospholipid side-chains. An aliquot of the phospholipid fraction equivalent to 12.5 g of soil dw was taken for PLFA analysis. After mild alkaline hydrolysis, the lipid extract was separated into OH-substituted ester-linked PLFAs, non-OH-substituted ester-linked PLFAs and unsaponifiable lipids following procedures described elsewhere (Zelles and Bai, 1993).

The fraction of unsubstituted ester-linked PLFAs was reduced to dryness under nitrogen and dissolved in 100 μl of hexane supplemented with nonadecanoic methyl ester as

internal standard. The analyses of the fatty acid methyl ester (FAME) extracts were performed by GC/MS as described previously (Lipski and Altendorf, 1997). The position of double bonds of monounsaturated fatty acids was determined by analysing the dimethyl disulfide (DMDS) adducts (Nichols *et al*., 1986). Quantification of 16:1Δcis8 and 18:1Δcis10 (signature PLFAs for methanotrophic bacteria; Bowman *et al*., 1993) was based on the abundance of characteristic ions of their DMDS adducts.

The fraction of unsaponifiable lipids was cleaved during acidic alkaline hydrolysis and the resulting non-ester-linked PLFAs were separated into OH-substituted non-ester-linked PLFAs (UNOH) and non-OH-substituted non-ester-linked PLFAs (UNSFA). Separation of the non-ester-linked PLFAs, derivatization and measurement were performed according to Gattinger and colleagues (2002b). Subgroups of UNSFA were named according to their functional groups: '-ant' (anteiso-branching), '-nor' (normal straight chain), '-uns' (unsaturations). The positions of double bonds were given from the carboxyl group of the fatty acid molecule according to the recommendations of the IUPAC-IUB Commission on biochemical nomenclature (IUPAC-IUB Commission on biochemical nomenclature, 1977). Another aliquot of the phospholipid fraction equivalent to 25.0 g of soil dw was used for PLEL analysis according to Gattinger and colleagues (2003). After the formation of ether core lipids, ether-linked isoprenoids were released following cleavage of ether bonds with HI and reductive dehalogenation with Zn in glacial acetic acid. The resulting isoprenoid hydrocarbons were dissolved in 100 μl of internal standard solution (nonadecanoic methyl ester) and subjected to GC/MS analysis at operating conditions described elsewhere (Gattinger *et al*., 2003). PLFA/PLEL concentrations are expressed in nmol g^{-1} dw.

Statistical analysis

Statistical analyses were carried out using Systat 10. Concentrations of the individual PLFAs and PLELs were subjected to PCA to elucidate major variation patterns. Functional subgroups of UNSFA and UNOH were included (see Zelles, 1999) in the PCA data set to ease interpretation of the PCA result as both fractions were compiled by 20–40 different single compounds (data not shown). There was no significant influence on the PCA results, if single compounds of UNSFA and UNOH or their functional subgroups were used.

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