Bacterial community structure and carbon turnover in permafrost-affected soils of the Lena Delta, northeastern Siberia[1]

Dirk Wagner, Svenja Kobabe, and Susanne Liebner

Abstract: Arctic permafrost environments store large amounts of organic carbon. As a result of global warming, intensified permafrost degradation and release of significant quantities of the currently conserved organic matter is predicted for high latitudes. To improve our understanding of the present and future carbon dynamics in climate sensitive permafrost ecosystems, the present study investigates structure and carbon turnover of the bacterial community in a permafrost-affected soil of the Lena Delta (72°22′N, 126°28′E) in northeastern Siberia. 16S rRNA gene clone libraries revealed the presence of all major soil bacterial groups and of the canditate divisions OD1 and OP11. A shift within the bacterial community was observed along the soil profile indicated by the absence of *Alphaproteobacteria* and *Betaproteobacteria* and a simultaneous increase in abundance and diversity of fermenting bacteria like *Firmicutes* and *Actinobacteria* near the permafrost table. BIOLOG EcoPlates were used to describe the spectrum of utilized carbon sources of the bacterial community in different horizons under in situ temperature conditions in the presence and absence of oxygen. The results revealed distinct qualitative differences in the substrates used and the turnover rates under oxic and anoxic conditions. It can be concluded that constantly negative redox potentials as characteristic for the near permafrost table horizons of the investigated soil did effectively shape the structure of the indigenous bacterial community limiting its phylum-level diversity and carbon turnover capacity.

Key words: active layer, soil organic carbon, microbial diversity, permafrost ecosystem.

Résumé : Le pergélisol situé dans les zones arctiques constitue une vaste réserve de carbone organique. Le réchauffement climatique accroît la dégradation du pergélisol, ayant pour conséquence une libération massive de matière organique prévue dans les régions de hautes latitudes. De manière à améliorer notre compréhension de la dynamique présente et future du carbone dans les écosystèmes climatiques sensibles, telles que le pergélisol, cette étude examine la structure et le turnover du carbone de la communauté bactérienne contenue dans le sol sous influence directe du pergélisol, dans le delta de la Lena (72°22′N, 126°28′E) au nord de la Sibérie. Le clonage du gène 16S rRNA a révélé la présence de la totalité des groupes importants de bactéries du sol, ainsi que des divisions présumées OD1 et OP11. Un changement au sein de la communauté bactérienne a été observé le long du profil de sol, indiqué par l'absence des *Alphaproteobacteria* et des *Betaproteobacteria* et l'augmentation simultanée de l'abondance et de la diversité des bactéries fermentatives, comme par exemple les *Firmicutes* et les *Actinobacteria*, à proximité du pergélisol. Des plaques BIOLOG Eco ont été utilisées sous conditions de température contrôlées et en présence ou absence d'oxygène, afin de décrire le spectre des sources de carbone utilisées par la communauté bactérienne dans les différents horizons du sol. Les résultats ont révélé des différences qualitatives distinctes concernant les substrats utilisés, ainsi que le turnover du carbone sous conditions oxiques et anoxiques. En conclusion, les potentiels d'oxydo-réduction exclusivement négatifs, caractérisant l'horizon du sol proche du pergélisol, façonnent efficacement la structure de la communauté bactérienne indigène, limitant sa diversité au niveau du phylum, ainsi que sa capacité à utiliser le carbone organique contenu dans le sol.

Mots-clés : couche active, carbone organique du sol, diversité microbienne, pergélisol.

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Introduction

Terrestrial permafrost is identified as one of the most vulnerable carbon pools of the Earth system (Osterkamp 2001; Zimov et al. 2006). About one-third of the global soil carbon is preserved in northern latitudes (Gorham 1991), mainly in huge layers of frozen ground, which underlay around 24% of the exposed land area of the northern hemisphere (Zhang et al. 1999). This carbon reservoir is of global climatic importance, in particular because of the currently observed climate changes in the Arctic (Intergovernmental Panel on Climate Change (IPCC) 2007).

The degradation of permafrost could release large quantities of previously frozen organic matter. Permafrost degrada-

tion through environmental changes is considered to have a stronger impact on organic carbon decomposition rates than the direct effect of temperature rise alone (Eugster et al. 2000). This process is associated with the release of climate-relevant trace gases from intensified microbial carbon turnover that may further increase global warming and transform the Arctic tundra ecosystems from a carbon sink to a carbon source (Oechel et al. 1993).

The Lena Delta in northeastern Siberia is located in the zone of continuous permafrost. Permafrost-affected soils of the Siberian Arctic are subjected to freezing and thawing cycles during the year, which lead to the formation of typical patterned grounds with a prominent microrelief. More than two-thirds of the Lena Delta are dominated by moist to wet ecosystems (Schneider et al. 2009); for example, low-centred ice-wedge polygons and shallow water areas where redox conditions are predominantly negative (Fiedler et al. 2004). Under these conditions, the mineralization of organic matter can only be realized stepwise by specialized microorganisms of the so-called anaerobic food chain (Schink and Stams 2006). Important intermediates of the organic matter decomposition under anaerobic conditions are polysaccharides, low molecular mass organic acids, phenolic compounds, and sugar monomers (Guggenberger et al. 1994; Kaiser et al. 2001). The fermentation of carbon by microorganisms does thereby run much slower than oxidative respiration. Thus, an increase of the abundance of water-saturated soils, as predicted for Arctic permafrost environments resulting from thermokarst erosion (Grosse et al. 2006), might be accompanied by qualitative and quantitative alterations within the turnover of organic matter.

Although microorganisms are the drivers of carbon mineralization, the structure of the microbial community and its influence on carbon dynamics and ecosystem stability in Arctic permafrost-affected soils remain poorly understood (Wagner 2008). Although several studies investigated the diversity of either, for example, methanogens (Høj et al. 2005, 2006; Ganzert et al. 2007) and methanotrophs (Kaluzhnaya et al. 2002; Wartiainen et al. 2003; Liebner et al. 2009) or of the entire microbial community (Zhou et al. 1997; Kobabe et al. 2004; Neufeld and Mohn 2005; Hansen et al. 2007; Steven et al. 2007), nothing is known about how microbial communities in Arctic regions react to site specific variations within the redox regime.

This study, therefore, provides a combined clone library and BIOLOG approach, and links an analysis of carbon turnover rates and substrate utilization with that of community structure and diversity along a water-logged, permafrost-affected soil with vertical alteration of redox conditions.

Materials and methods

Site description and soil sampling

The investigation site is located on Samoylov Island (72°22′N, 126°28′E), in the central part of the Lena Delta, northeastern Siberia. A detailed description of the geomorphologic situation of the island and the whole delta was given previously (Schwamborn et al. 2002; Wagner et al. 2003). The Lena Delta is located in the zone of continuous permafrost and is characterized by an Arctic continental climate with a mean annual air temperature of –14.7 °C (T_{min} = –48 °C, T_{max} = 18 °C) and a mean annual precipitation of about 190 mm. The landscape of the delta is dominated by a microrelief of ice-wedge polygons, which develop because of the annual freeze–thaw cycles. The soils are totally frozen for at least 8 months every year, and only a shallow active layer of about 20–50 cm soil depth is unfrozen during the summer months. The active layer of the polygon is characterized by a steep temperature gradient from ~10 °C in the top horizon to almost 0 °C near the permafrost table (Wagner et al. 2003). One of the main landscape formations is the low-centred polygon, where the flat central parts are surrounded by raised rims. The investigated soils in the polygon centres are characterized by a water level near the soil surface, which together with the cold climate conditions leads to an accumulation of organic matter and formation of peat layers. The mainly anaerobic decomposition of soil organic matter in combination with the water regime and the vegetation generates high CH_4 production and emission rates from these sites (Wille et al. 2008). The vegetation of the polygon centre is dominated by hydrophytes like various *Carex* species and different moss species (e.g., *Limprichtia revolvens* (Sw.) Loeske and *Meesia longiseta* Hedw.; Kutzbach et al. 2004).

Soil sampling was carried out in the summers of 2002 and 2005 during Russian–German expeditions LENA 2002 and 2005 on Samoylov Island. We defined our sampling profiles according to soil horizons following Schoeneberger et al. (2002). As bacteria are associated with mineral and organic soil particles (Christensen et al. 1999), it is reasonable to assign microbial communities to soil horizons. The profile at the centre of a low-centred polygon was located in the eastern part of the island. At the time of soil sampling, the standing water level was at a depth of ~10 cm above the surface. The depth of the permafrost table was at 45 cm. Soil samples were taken from each horizon, stored in Nalgene boxes, frozen immediately after sampling, and transported to Germany for further processing.

Extraction of total DNA

Near-surface and near-permafrost table samples of the polygon centre were thoroughly homogenized and dispersed into 6 subsamples of 0.5 g. Four subsamples of each depth were used for extraction of total DNA. The remaining subsamples were used for fluorescence in situ hybridization (FISH). Total genomic DNA was extracted with the BIO 101 Fast DNA SPIN Kit for Soil (Qbiogene, Inc.). Quality and quantity of the genomic DNA was analysed by electrophoresis on a 1% agarose gel against a Lamda *Eco*RI/*Hin*dIII marker (ABgene) with ethidium bromide staining.

PCR amplification

PCR amplification reactions were performed with a thermal cycler (iCycler; Bio-Rad Laboratories). PCR reaction mixes (25 μL) contained 1× PCR reaction buffer, 0.2 μmol/L primer, 0.25 μmol/L dNTPs, 1.25 U MasterTaq polymerase (Eppendorf), and 0.6–160 ng of template. The universal bacterial primers GM3 (5′-AGAGTTTGATCMTGGC-3′) targeting *Escherichia coli* position 8–24 and GM4 (5′-TACCTTGTTACGACTT-3′) targeting *E. coli* position 1492–1507 were used for amplification of nearly the whole bac-

terial 16S rRNA gene (Muyzer et al. 1995). PCR conditions were as follows: 5 min at 94 °C (initial denaturation), followed by 20 cycles of 1 min at 94 °C, 1 min at 42 °C (annealing), and 3 min at 72 °C, and by a final elongation at 60 °C for 60 min. PCR products (in 96-well plates, ABgene) were purified by centrifugation (980*g* at 4 °C) through Multi Screen 96-well plates (Millipore) on a column of Sephadex G-50 Superfine powder (GE Healthcare Bio-Science).

Construction of clone libraries and sequencing

Purified PCR products were ligated into the linear Plasmid Vector pCR4 supplied with the TOPO TA Cloning Kit for Sequencing (Invitrogen) and subsequently transformed into chemically competent *E. coli* cells via heat shock following the manufacturer's protocol. Cells were incubated overnight at 37 °C on agar plates containing 0.05% ampicillin. Colonies were screened by PCR with vector primers for the correct size of the insert, and the amplicons were directly sequenced. Sequencing was performed with the BigDye Terminator cycle sequencing kit, version 3.1 (Applied Biosystems), with vector primers.

Sequence analysis

Sequences were edited with Sequencing Analysis 5.2 (Applied Biosystems) and checked for chimeras with Bellerophon (Huber et al. 2004) and with the Chimera-Check of the Ribosomal Database Project (Michigan State University). Assembly of contigs was performed with Sequencher 4.7 (Gene Codes). Sequences were imported into ARB (available from www.arb-home.de and www.arb-silva.de) and phylogenetically analyzed. Only sequences with >700 nucleotides were used. Sequences were assigned to operational taxonomic units (OTUs) to compare the number of species near the surface and near the permafrost table. 16S rRNA gene sequences with ≥97% similarity were considered as 1 OTU.

Nucleotide Accession Nos.

The 16S rRNA gene sequences were deposited in the EMBL, GenBank, and DDBJ nucleotide sequence databases under Accession Nos. EU644280–EU644346 (Centre_near permafrost table), and EU644347–EU644445 (Centre_near surface).

BIOLOG plate preparation

BIOLOG Eco microtiter plates (EcoPlates; Biolog Inc., Hayward, California) were used to describe the spectrum of utilized carbon sources of the microbial community in different horizons of the polygon centre under oxic and anoxic conditions. Therefore, EcoPlates were used providing intraplate replication, as each 96-well plate contains 31 different carbon substrates and 1 additional blank in a 3 triplicate array according to Insam (1997). Microorganisms were extracted from 10 g of soil with 90 mL of sterile Ringer solution (0.25%). Soil suspensions were then shaken for 30 min on a reciprocal shaker. After the extraction of microorganisms, the cell density was calculated by direct cell counts of dichlorotriazinyl aminofluorescein (DTAF)-stained samples as described previously (Kobabe et al. 2004). Afterwards each EcoPlate well was inoculated with 125 µL of a suspension with a cell density of 5×10^7 cells·mL^{-1} and incubated under aerobic and anaerobic conditions at 5 °C in darkness. For the substrate test under anaerobic conditions all preparatory steps including the incubation were done in a glove box under an atmosphere of pure nitrogen.

Plate reading and data analysis

The absorbance of each plate at 595 nm was measured at time intervals on a plate reader (SLT Spectra II Classic). Absorbance values for the wells with carbon sources at a similar average well colour development (AWCD) of 0.2 ± 0.01 OD were taken and were blanked against the control wells at $t = 0$. Negative values were considered as 0 in subsequent data analyses. The AWCD values at 0.2 ± 0.01 OD were reached, on average, after 98 h (63–133 h) in the presence of oxygen and after 162 h (135–190 h) in the absence of oxygen, depending on the substrate turnover rate of the different horizons. Finally, standardization of the data was performed according to Garland and Mills (1991) by dividing each OD value by the AWCD.

Results of the absorbance measurements of the individual EcoPlates were subjected to principal component analyses (PCA). PCA is an eigenvector analysis procedure (details described by Glimm et al. (1997)) that attempts to maximize the amount of variance in the data set that can be explained by the first few principal components. Analyses of variance (ANOVA) were performed for each axis of the PCA. Subsequent to the ANOVA test, significant differences in the microbial communities were analysed with the Fisher LSD test (in the case of variance heterogeneity) or by the Scheffé test (in the case of variance homogeneity; Glimm et al. 1997). All statistical analyses were performed using Statistica 6.1 (StatSoft, Inc.).

Fluorescence in situ hybridization (FISH)

FISH was performed on subsamples of the 2 depths of the polygon centre to obtain a quantitative complementation of the clone library analysis. Probes targeting main soil bacterial groups were used (Table 1). Fixation of soil samples, hybridization, DAPI staining, and determination of cell counts were carried out as described elsewhere (Liebner and Wagner 2007).

Results

Structure and diversity of the bacterial community

Based on 178 sequences with >700 nucleotides, a total of 137 OTUs could be assigned within the polygon centre, which were affiliated to 11 phyla and subphyla. These included *Alpha-*, *Beta-*, and *Deltaproteobacteria*, *Bacteroidetes*, *Chlorobi*, *Verrucomicrobia*, *Planctomycetes*, *Acidobacteria*, *Firmicutes*, *Actinobacteria*, and *Thermomicrobia*. In addition, the candidate divisions OD1 and OD11 were identified. The relative abundance and affiliation of the 16S rRNA gene sequences retrieved from the near-surface and the near-permafrost-table horizons of the polygon centre are summarized in Fig. 1 and Table 2. According to the results of the clone library, the number of bacterial groups and OTUs decreased towards the permafrost table. While all bacterial groups detected in the soil were present near the surface, *Alphaproteobacteria* and *Betaproteobacteria*, as well as *Planctomycetes* and OD1, were not identified

Table 1. rRNA-targeting oligonucleotide probes used for fluorescence in situ hybridization (FISH) of soil samples from Samoylov Island, Lena Delta, northeastern Siberia.

Probe	Target group	Sequence (5′–3′) of probe	Target site[a]	FA (%)[b]	Reference
EUB338	Domain *Bacteria*	GCTGCCTCCCGTAGGAGT	16S rRNA (338)	0–35	Amann et al. (1990)
EUB338 II	Domain *Bacteria*	GCAGCCACCCGTAGGTGT	16S rRNA(338)	0–35	Daims et al. (1999)
EUB338 III	Domain *Bacteria*	GCTGCCACCCGTAGGTGT	16S rRNA(338)	0–35	Daims et al. (1999)
NON338	Complementary to EUB338	ACTCCTACGGGAGGCAGC	16S rRNA	nd	Wallner et al. (1993)
ALF968	α-Subclass of *Proteobacteria* (except *Ricketsiales*)	GGTAAGGTTCTGCGCGTT	16S rRNA(968)	35	Neef (1997)
Bet42a	β-Subclass of *Proteobacteria*	GCCTTCCCACTTCGTTT	23S rRNA (1027)	35	Manz et al. (1992)
Gam42a	γ-Subclass of *Proteobacteria*	GCCTTCCCACATCGTT	23S rRNA (1027)	35	Manz et al. (1992)
CF319a[c]	Most *Flavobacteria*; some *Bacteroidetes* and *Sphingobacteria*	TGGTCCGTGTCTCAGTAC	16S rRNA (319)	35	Manz et al. (1996)
CF319b	Same as for CF319a	TGGTCCGTATCTCAGTAC	16S rRNA (319)	35	Manz et al. (1996)
CFB719[c]	Most *Bacteroidetes*; some *Flavobacteria* and *Sphingobacteria*	AGCTGCCTTCGCAATCGG	16S rRNA (719)	30	Weller et al. (2000)
HGC69a	*Actinobacteria* (Gram-positive bacteria with high G+C content)	TATAGTTACCACCGCCGT	23S rRNA (1901)	25	Roller et al. (1994)
LGC354a	*Firmicutes* (Gram-positive bacteria with low G+C content)	TGGAAGATTCCCTACTGC	16S rRNA (354)	35	Meier et al. (1999)
LGC354b	Same as for LGC354a	CGGAAGATTCCCTACTGC	16S rRNA (354)	35	Meier et al. (1999)
LGC354c	Same as for LGC354a	CCGAAGATTCCCTACTGC	16S rRNA (354)	35	Meier et al. (1999)

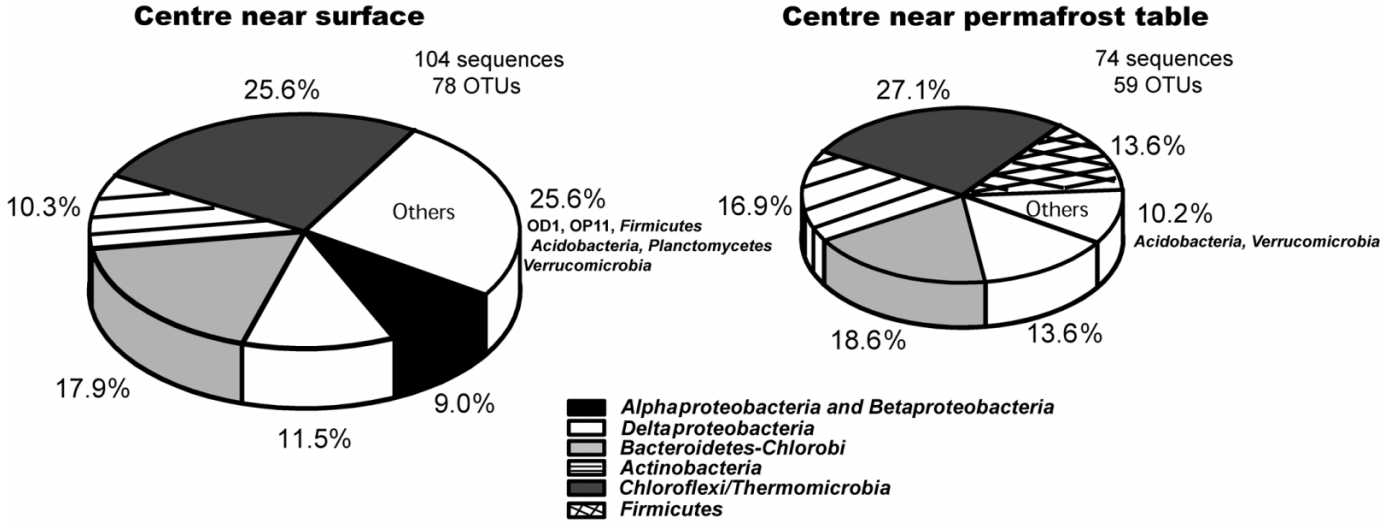
Note: nd, not determined.

[a]*Escherichia coli* numbering.

[b]Percentage (*v*/*v*) of formamide in the hybridization buffer.

[c]A combination of these probes detected 71.2% of all *Bacteroidetes-Chlorobi* cells of this study (according to the Probe Match function in the ARB software).

Fig. 1. Relative abundance and affliation of bacterial operational taxonomic units (OTUs) in a low-centred polygon on Samoylov Island, Lena Delta, northeastern Siberia.



near the permafrost table. In return, the communities of *Actinobacteria* and, in particular, of *Firmicutes* increased in diversity near the permafrost table, yielding a higher number of representatives of the orders *Clostridiales*, *Actinomycetales*, and *Rubrobacterales* (Table 2).

The phylogenetic analysis is consistent with the quantification of cells through FISH (Table 3). Although the probe mix targeting *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* generated positive results in both depths, the amount of cells detected through this mix in relation to all *Bacteria* affiliated cells decreased from 5.3% near the surface to 1.2% near the permafrost table. In contrast, cell counts of high guanine and cytosine (GC) Gram-positive bacteria (e.g., *Actinobacteria*) and low GC Gram-positive bacteria (e.g., *Firmicutes*) relative to all *Bacteria* affiliated cells significantly increased with depth. Gram-positive bacterial cells with high GC contents contributed 19.6% to all bacteria near the surface and 26.7% to all

Table 2. Phylogenetic affiliation of 16S rRNA gene sequences derived from a polygon centre on Samoylov Island, Lena Delta, northeastern Siberia.

Phylogenetic affiliation	Genus or closest cultured relative	No. of clones	
		Near surface	Near permafrost table
Proteobacteria			
Alphaproteobacteria		5	
Sphingomonadaceae	*Sphingomonas*	3	
Rhodospirillaceae	*Azospirillum*	1	
Unaffiliated		1	
Betaproteobacteria		3	
Comamonadaceae	*Polaromonas*	1	
Gallionellaceae	*Gallionella*	2	
Deltaproteobacteria		10	8
Syntrophaceae	*Syntrophus*	4	4
Geobacteraceae	*Geobacter propionicus*	1	1
	Geobacter bremensis	3	1
Unaffiliated		2	2
Bacteroidetes-Chlorobi		31	18
Bacteroidaceae	*Bacteroides*	1	
Sphingobacteriaceae	Unaffiliated	26	16
Unaffiliated *Chlorobi*		4	2
Verrucomicrobia		2	2
Verrucomicrobiaceae	*Prosthecobacter*	2	2
Planctomycetes		5	
Planctomycetaceae	*Pirellula*	3	
Unaffiliated *Planctomycetales*	*Candidatus Kkuenenia*	2	
Acidobacteria		3	4
Group 6			4
Group 8	*Holophaga*	3	
Firmicutes		3	11
Clostridiales	*Propionispira*	1	2
	Sporotalea		2
Clostridiaceae	*Acetivibrio*		4
	Clostridium	2	1
Unaffiliated			2
Actinobacteria		10	12
Unaffiliated *Actinobacteria* (class)	*Microthrix*		1
Conexibacteraceae	*Conexibacter*		4
Cellulomonadaceae	*Cellulomonas*		3
Intrasporangiaceae	Unaffiliated	3	
Propionibacteriaceae	*Propionibacterium*	3	2
Rubrobacteraceae	*Rubrobacter*	1	
Unaffiliated		3	2
Chloroflexi		22	17
Unaffiliated *Thermomicrobia*		13	9
Unaffiliated *Chloroflexi*		9	8
OD1		4	
OP11		6	2
Total number of sequences		104	74

bacteria near the permafrost table. Gram-positive bacterial cells with low GC content contributed 12.7% to all bacteria near the surface and 20.9% to all bacteria near the permafrost table.

Substrate metabolisms under oxic and anoxic conditions

For comparison of the physiological potential of the microbial communities in the different horizons to degrade various substrates, the results of carbon turnover in the BIOLOG EcoPlates were subjected to PCA (Fig. 2). The turnover rates of the samples were compared at an early stage of incubation (AWCD 0.2) to avoid growth of the microorganisms with the offered substrates. Under aerobic conditions the upper soil layer (0–5 cm) and the soil layer between 30 and 35 cm differed significantly ($p < 0.01$) from each other and from all other soil horizons, whereas the other soil depths could not be differentiated from each other by their substrate turnover (Fig. 2*a*). In the absence of

Table 3. Total and cell counts of various soil bacterial groups in a low-centred polygon on Samoylov Island, Lena Delta, northeastern Siberia, obtained through DAPI staining and fluorescence in sity hybridization (FISH).

	Counts ($\times 10^7$ cells·g^{-1})											
			CFB group									
			CF319a/b		CFB719		*Proteobacteria*[c]		High GC		Low GC	
Site	Total[a]	*Bacteria*[b]	Cell count	Relative to *Bacteria* (%)	Cell count	Relative to *Bacteria* (%)	Cell count	Relative to *Bacteria* (%)	Cell count	Relative to *Bacteria* (%)	Cell count	Relative to *Bacteria* (%)
Near surface	26.4±7.00	9.19±1.24	0.25±0.06	4.6	0.69±0.35	7.5	0.48±0.01	5.3	1.8±0.04	19.6	1.8±0.01	12.7
Near permafrost table	8.28±3.35	3.9±0.98	0.82±0.12	22.9	0.5±0.04	13.9	0.04±0.008	1.2	0.96±0.13	26.7	0.76±0.15	20.9

Note: All values are displayed as means ± SDs. GC, guanine and cytosine; CFB, *Cytophaga-Flavobacter-Bacteroides*.

[a]Obtained through DAPI staining.

[b]Probe mix of EUB338 I, II, and III.

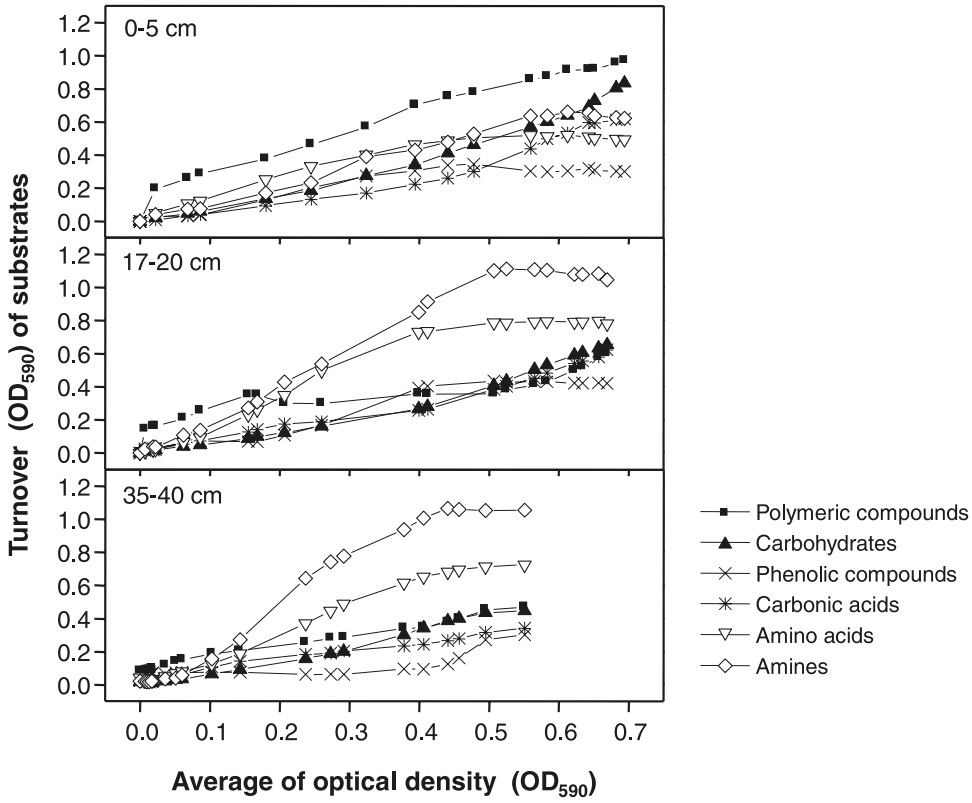
[c]Probe mix of ALF968, Bet42a, and Gam42a.

Fig. 2. Principal component (PC) diagram of substrate utilization in the different horizons incubation under (*a*) aerobic and (*b*) anaerobic conditions at average well colour development (AWCD) 0.2. The metabolism of the various substrates was determined using BIOLOG EcoPlates. The numerals designate the different sampling depths of each horizon. Values are means ± SEs (n = 3).

oxygen, the upper 4 soil layers (0–20 cm) were significantly different ($p < 0.05$) from each other and from the deeper soil horizons (Fig. 2*b*). In contrast to these upper soil horizons, the deeper layers (20–45 cm) were not significantly different from each other.

On closer examination of the substrate turnover in the different horizons on basis of the 6 major substance classes (amines, amino acids, carbonic acids, carbohydrates, phenolic compounds, and polymers) proposed by Insam (1997), it was shown that under aerobic conditions the upper soil layer (0–5 cm) was distinctly different from all other horizons by the preferred substrates converted. Here, polymeric substances (e.g., α-cyclodextrin and glycogen) and carbohydrates (e.g., D-cellobiose and glucose-1-phosphate) were better degraded than in the other horizons. In contrast with the upper soil horizon, amines and amino acids were degraded with the highest rate (L-asparagine and L-serine were the highest) in all other soil layers. Figure 3 shows the turnover rates in the presence of oxygen of the different chemical classes for 3 soil depths (upper, middle, and bottom soil

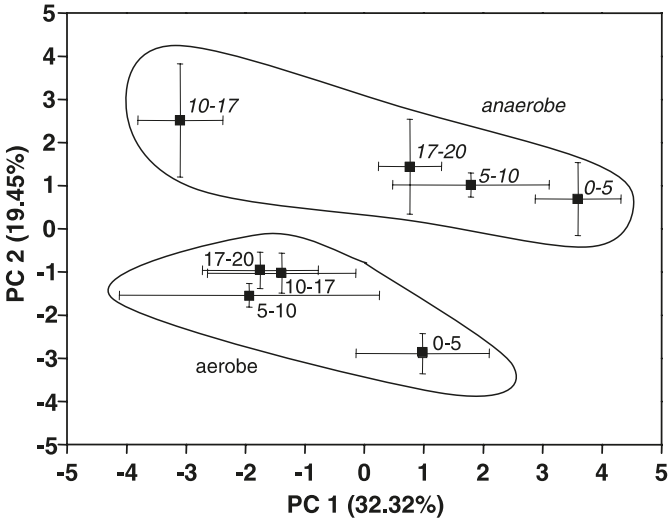
Fig. 3. Turnover of the different substrates represented by their chemical guild under aerobic conditions in 3 selected horizons of the soil from the polygon centre. The metabolism of the various substrates was determined using BIOLOG EcoPlates.



layers). The substrate turnover of the other horizons was similar to the middle (17–20 cm) and bottom (35–40 cm) soil layers (results are not shown). Under anaerobic conditions the turnover was slower, but in contrast with aerobic decomposition, polymers and carbohydrates were more rapidly degraded in all horizons (results are not shown).

The upper part of the active layer in the polygon centre is influenced by varying oxygen concentrations owing to changes in the water table during the vegetation period. To discover any differences between aerobic and anaerobic decomposition of organic substrates in this zone, the data obtained from both oxic and anoxic incubation for the upper 20 cm of the soil were subjected to PCA (Fig. 4). The analysis showed that the substrate turnover was significantly different (p = 0.05) under the different redox conditions in all horizons. Aerobic and anaerobic substrate decomposition were mainly differentiated by the turnover of L-serine, which degraded much better in the presence of oxygen (Table 4). Furthermore, D-cellobiose and α-cyclodextrin degraded better under oxic conditions, whereas pyruvic acid methyl ester and putrescine degraded better under anoxic conditions.

Fig. 4. Principal component (PC) diagram of substrate metabolism in comparions of aerobic and anaerobic conditions for the first 20 cm of the soil profile. The numerals designate the different sampling depths of each horizon. Values are means ± SEs (n = 3).



Discussion

The great extent of moist to wet tundra sites indicates the importance of wetlands for the carbon dynamics of ecosystems in the Lena Delta. Coverage by wet sites might temporarily increase in the future because of the degradation of permafrost and thermokarst erosion (Grosse et al. 2006). The consequences of carbon turnover thereby strongly depend on the structure and physiology of the indigenous microbial communities within the active layer and the underlying perennially frozen ground. The combined clone library and BIOLOG approach of this study revealed a vertical shift in carbon turnover rates and in the utilization of substrates in samples of a polygonal peat soil, which corresponded to changes in bacterial community structure and diversity.

Table 4. Comparison of the different substrates of the BIOLOG EcoPlates under oxic and anoxic conditions according to their eigenvector at average well colour development (AWCD) 0.2 for axis 2.

Substrate	Substance class	Eigenvector
Methyl pyruvate	Carbonic acid	0.581
Putrescine	Amine	0.472
Glucose-1-phosphate	Carbohydrate	0.440
Tween 80	Polymer	0.430
N-Acetyl-D-glucosamine	Carbohydrate	0.413
Itanoic acid	Carbonic acid	0.404
β-Methyl-D-glucoside	Carbohydrate	0.380
D-Malic acid	Carbonic acid	0.370
Tween 40	Polymer	0.243
D,L-α-Glycerol phosphate	Carbohydrate	0.228
D-Galactonic acid lactone	Carbohydrate	0.191
L-Phenylalanine	Amino acid	0.189
L-Asparagine	Amino acid	0.127
D-Mannitol	Carbohydrate	0.087
Glycyl-L-glutamic acid	Amino acid	0.085
iso-Erythritol	Carbohydrate	0.076
α-Ketoglutaric acid	Carbonic acid	0.023
D-Galacturonic	Carbonic acid	–0.156
4-Hydroxybenzoate	Phenolic compound	–0.215
Xylose	carbohydrate	–0.268
L-Arginine	Amino acid	–0.309
Phenyl ethylamine	Amine	–0.310
2-Hydroxybenzoate	Phenolic compound	–0.349
α-D-Lactose	Carbohydrate	–0.350
L-Threonine	Amino acid	–0.383
D-Glucosaminic acid	Carbonic acid	–0.396
Glycogen	Polymer	–0.423
γ-Hydroxybutyric acid	Carbonic acid	–0.453
α-Cyclodextrin	Polymer	–0.558
D-Cellobiose	Carbohydrate	–0.661
L-Serine	Amino acid	–0.928

Changes of the bacterial communities along the soil profile are accompanied by small-scale temporal and spatial variation in temperature regime and water balance (Boike et al. 2008). The ability of *near-surface* microbial communities to differently respond to aerobic and anaerobic conditions with higher turnover rates in the presence of oxygen (Fig. 4) thereby reflects seasonal alterations of in situ redox conditions characteristic of the uppermost active layer horizons of the polygon centre (Fiedler et al. 2004). Enlarged redox potentials in the top layers of the prevailing water-saturated polygon centre result, for example, from plant-mediated oxygen transport into the rhizosphere (Kutzbach et al. 2004) or seasonal variations of the water level (Wagner et al. 2003). Representatives of *Sphingomonas* and *Azospirillum* (*Alphaproteobacteria*) and of *Polaromonas* and *Gallionella* (*Betaproteobacteria*) were only detected near the surface. These organisms respire organic material with oxygen as the electron acceptor (e.g., see Tarrand et al. (1978), Takeuchi et al. (2001), Hanert (2006), and Kämpfer et al. (2006)). They all possess ubiquinones in their respiratory chains that cause the more rapid degradation of the various substrates offered during the incubation experiments (e.g., carbohydrates and polymeric compounds) in comparison with the deeper horizons. These results are consistent with other studies reporting that soil respiration (expressed as soil CO_2 production) rose exponentially with increasing redox potential (Yu et al. 2007). Still, switching to an anaerobic milieu did not cut off, but only slowed down, carbon turnover of the substrates offered in the BIOLOG plates accompanied by a shift in the utilized substrates (Fig. 3). Fermenting bacteria such as *Propionispira*, *Clostridium* (*Firmicutes*), and *Propionibacterium* (*Actinobacteria*) could have taken over carbon utilization under these conditions. Thus, the environmental conditions, in particular the fluctuating redox potentials, indicative of the near surface of the peat soil in the polygonal centre, permit a diverse community of bacteria that are able to utilize a wide spectrum of substrates to be established. This community can rapidly turn over carbon under aerobic conditions, but keeps active also under oxygen limitation.

In contrast with the near surface, redox conditions of the *near-permafrost* horizon of the polygon centre are constantly negative because of its permanent water saturation (Fiedler et al. 2004). This is likely to limit growth and activity of obligate aerobes. Although *Sphingobacteriaceae*, which are predominantly aerobes (e.g., Shivaji et al. 1991; Steyn et al. 1998; Kim and Jung 2007), are abundant and diverse in the bottom zone of the active layer, other aerobes occurring near the surface were not detected near the permafrost table on the basis of our clone libraries. These are representatives of *Alphaproteobacteria* and *Betaproteobacteria* and *Pirellula* (*Planctomycetes*), an obligate aerobic heterotroph. In addition, specific cell numbers obtained by FISH revealed that the proportion of *Alphaproteobacteria* and *Betaproteobacteria* compared with all bacterial cells declined greatly towards the permafrost table. Combined with an increase in diversity of fermenting bacteria, in particular within the groups of *Firmicutes* and *Actinobacteria*, we assume a specialization of the bacterial community near the permafrost table to the constantly anaerobic environment indicated by the low redox potentials. Absence of the ability to respire organic substrates faster in the presence of oxygen, as shown in the BIOLOG plate experiments, points to a lack of organisms near the permafrost table, which are adapted to high redox conditions. Such a response in community structure might explain that the utilization of substrates provided by the BIOLOG plates is restricted near the permafrost table compared with the near surface. This is plausible as the soil organic carbon in the upper soil horizon comprises greater than two-thirds of the undecomposed light-carbon fraction (Gundelwein et al. 2007), which offers a large spectrum of organic carbon compounds. In contrast, the light-carbon fraction is mainly decomposed and therefore less biodegradable near the permafrost table (Wagner et al. 2005).

A specialization in terms of physiological properties and community structure as observed for the polygon centre soil might not only be necessary based on the redox regime or the concentration of oxygen, respectively, alone, but the specialization may also be necessary considering that organic substrates are less available for microorganisms with increasing soil depth. This results from a pronounced accumulation of organic matter (peat formation) owing to the

continuously anaerobic conditions and low in situ temperatures. It was demonstrated that the availability of organic carbon in permafrost soils decreased with increasing humification index (HIX, dimensionless) and soil depth (Wagner et al. 2005). HIX is a criterion for organic matter quality and can, therefore, give suitable information with regard to microbial metabolism (Zsolnay 2003), as the amount of organic carbon alone gives no information on the quality and availability of compounds as energy and carbon sources for microorganisms (Wagner and Liebner 2009 and references therein).

Our study revealed distinct differences in both the quality of substrates used in the different horizons and the rates of substrate turnover under aerobic and anaerobic conditions. As expected, the substrate turnover under anoxic conditions was, in general, much slower than under oxic conditions. These differences could be mainly attributed to the preferential turnover of polymers and carbohydrates in the presence of oxygen, whereas in the absence of oxygen, amines and carbonic acids were favourably degraded by the soil microbial community. This is in accordance with results by Gundelwein et al. (2007), who reported strong vertical differentiation into distinct decomposition zones that was dependent on water saturation of a permafrost-affected soil from the Taimyr Peninsula, northern Siberia. An unexpectedly large portion (between 60% and 90%) of the soil carbon was found to be part of the passive carbon fraction, particularly in the bottom horizons of the active layer. The lignin content, for instance, increased from 33% in the top soil to 46% in the near-permafrost horizon. The degradation of lignin results in products such as polysaccharides, low molecular mass organic acids, phenolic compounds, and sugar monomers (Guggenberger et al. 1994; Kaiser et al. 2001), representing substrates that were provided in the used BIOLOG EcoPlates (Insam 1997). After ~1 year, however, a large fraction of lignin was transformed into less decomposable lignin polymers (Bahri et al. 2008).

Conclusions

It is known that organic carbon turnover under anaerobic conditions occurs much slower than in the presence of oxygen. Also, depending on the redox conditions, different electron acceptors are utilized. Whether, however, microbial communities could potentially switch between different redox and substrate conditions thereby maintaining their carbon turnover capacity is not known. In the present study on a polygonal peat soil representative for wet, permafrost-affected tundra sites, we found strong indications that constantly negative ambient redox potentials did effectively shape the structure of the indigenous bacterial community limiting its diversity and carbon turnover capacity. It is tempting to predict that an increase and retention of water logged sites within the tundra environments of the Lena Delta would not only persistently favour anaerobic organic carbon turnover and through this also the formation of the climate-relevant trace gas methane. It would also yield a less flexible bacterial community limited in its physiological skills.

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