Scientific Report for Exchange Visit within the ESF MOLTER Research Program

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Purpose of the visit

During the last decades (1960 – 1990) permafrost soils have experienced increasing temperatures, e.g., in Eastern Siberia of approximately 1°C at depths between 1.6 m and 3.2 m and in Southern Norway 0.5 – 1.0°C in about three meters depths (Lemke et al. 2007). Permafrost soils are a big reservoir of below-ground organic carbon; they contain approximately 50% of the global amount (Kuhry et al. 2009). Thus, increasing soil temperatures lead to degradation of permafrost, an enhanced microbial decomposition of organic matter and therefore to a higher release of carbon to the atmosphere (Dutta et al. 2006; Schuur et al. 2009).

The understanding of biogeochemical processes, stabilization and degradation of organic matter in permafrost soils is the focus of my PhD project at the University of Cologne, Germany. Microbial activity is a key in the carbon cycle due to the microbial degradation and mineralisation of dead organic matter in soils and sediments. Phospholipid fatty acids (PLFAs) are an instrument to determined microbial communities as they are essential membrane components of living cells (Zelles 1999). After cell death they are degraded

rapidly thus, they are assumed to reflect the amount of living organisms (Denef et al. 2007). An advantage of the PLFA analysis is the independence of microbial cultivation in laboratories with which only about 1% of the microorganisms can be determined (Gattinger 2001). In combination with statistical analysis and ^{13}C measurements their metabolic activities can be identified (e.g., aerobic / anaerobic decomposition of organic matter; Becker-Heidmann & Scharpenseel 1992). Furthermore, more specific information on organic substrates degraded (young/old carbon pools) by soil microbes is gained by using compound-specific radiocarbon analysis (CSRA) of single PLFAs

The exchange visit at Ghent University, Belgium, had the intension to apply PLFA analysis to permafrost soils from two different regions in the high Arctic thereby learning lipid extraction and purification methods and stable carbon isotope analysis of individual compounds. Furthermore, PLFAs were extracted from selected soil samples in sufficient quantities for CSRA that I want to perform in the following month in my home laboratory.

Work carried out during the visit

During the visit, PLFA analysis was done on 22 soil samples from Svalbard, Norway (6 samples) and from the Lena-Delta, Russia (16 samples). Analyzed samples from Svalbard were from two different sites in a small, heterogeneous catchment near Ny-Ålesund (Upper Drainage Area - 3 samples and Kohlhaugen Hill - 3 samples). Within the Lena-Delta samples came from four different sites, from the Island Tit-Ari (4 samples) and from Samoylov Island (Samoylov Cliff - 5 samples, Fish Lake Polygon Rim - 4 samples, Fish Lake Polygon Centre - 3 samples).

The soil samples were extracted using phosphate-buffer/chloroform/methanol in a 0.9:1:2 ratio. Down to a soil depth of 60 cm 10 g material was extracted (except one sample which was done as a test) while from greater depth 20 g soil were used for extraction. The total lipid fraction which was gained in the chloroform phase was separated on a solid phase extraction (SPE) column of silica gel into glycol- , neutral- and phospholipids with chloroform, acetone and methanol respectively. The phospholipids were methylated by mild alkaline esterfication with methanolic KOH to form fatty acid methyl esters (FAMES) which were analyzed by capillary gas chromatography–combustion-isotope ratio mass spectrometry (GC–c-IRMS) (GC-C/DeltaPLUS XP Thermo Scientific). Identification of PLFAs was done using an internal standard (FAMEs 12:0 and 21:0) which was added to the samples shortly before the measurement. Every sample was measured twice to ensure reliable data. For calculation the average peak areas and average $\delta^{13}C$ values were used.

Furthermore, large amounts of material were extracted from two samples from the Fish Lake Polygon Rim, Lena-Delta (220 g and 210 g). These PLFAs will be separated into individual PLFAs on a preparative GC after the visit at the University of Cologne for the analysis of compound specific ^{14}C concentrations.

Main results

1) Overview of study areas

The two study areas, Lena-Delta and Svalbard, differ in their types of soil, vegetation, values of total organic carbon (TOC) and thickness of active layer (seasonal thawed layer of permafrost soil) (table 1).

Table 1: Overview of the study areas Lena-Delta and Svalbard

Parameter	Lena-Delta	Svalbard
coordinates	72°22' N 126°48' E	78°55' N 11°51' E
type of soil	sand to silt	mostly sand
vegetation	moss and grass (Island Tit-Ari: some small trees)	moss and less grass
content of total organic carbon (TOC)	0.8 – 10.0 wt-%	0.1 – 3.3 wt-%
active layer thickness	Ø 33 cm	70 cm
sampled layers	active layer and permafrost	active layer

Furthermore, they differed in number and concentrations of determinate phospholipid fatty acids. In total 18 different PLFAs were determined for Svalbard soils and 24 different compounds in soils from the Lena-Delta. The concentrations of total PLFAs varied between 0.05 and 2625 nmol PLFA C / g soil in all samples. PLFA concentrations of the Lena-Delta samples (0.11 -2725 nmol PLFA C / g soil) were significant higher (ANOVA $p < 0.05$) than in samples from Svalbard (0.05 – 46.2 nmol PLFA C / g soil). In addition, samples from the two different sample regions differed in their content of organic (table 1). This result indicated higher amount of microbial biomass for organic richer soils.

2) Study area Lena-Delta

2.1) Soil profiles containing active layer and permafrost layer

In figure 1 the distribution of five selected PLFAs is shown for depths profiles of three sampling sites with mainly samples from the active layer within the Lena-Delta. The selection was made due to their different biomarker functions, i.e. different microbial groups, and their occurrence in sufficient amounts at all sampling sites. The saturated PLFAs iso-15:0 and anteiso-17:0 are biomarkers for gram-positive bacteria (Waldrop & Firestone 2004). Gram-negative bacteria are indicated by PLFA 18:1w7c, fungi by PLFA 18:1w9c (Waldrop & Firestone 2004) and actinomycetes by PLFA 10Me18:0 (Bossio & Scow 1998).

In general PLFA concentrations in samples from Fish Lake Polygon Centre and Rim (average of 20.1 and 16.1 nmol PLFA C / g soil) were low compared to concentrations from Tit-Ari (average of 34.1 nmol PLFA C/g soil). This result indicated a higher microbial activity for Island Tit-Ari.

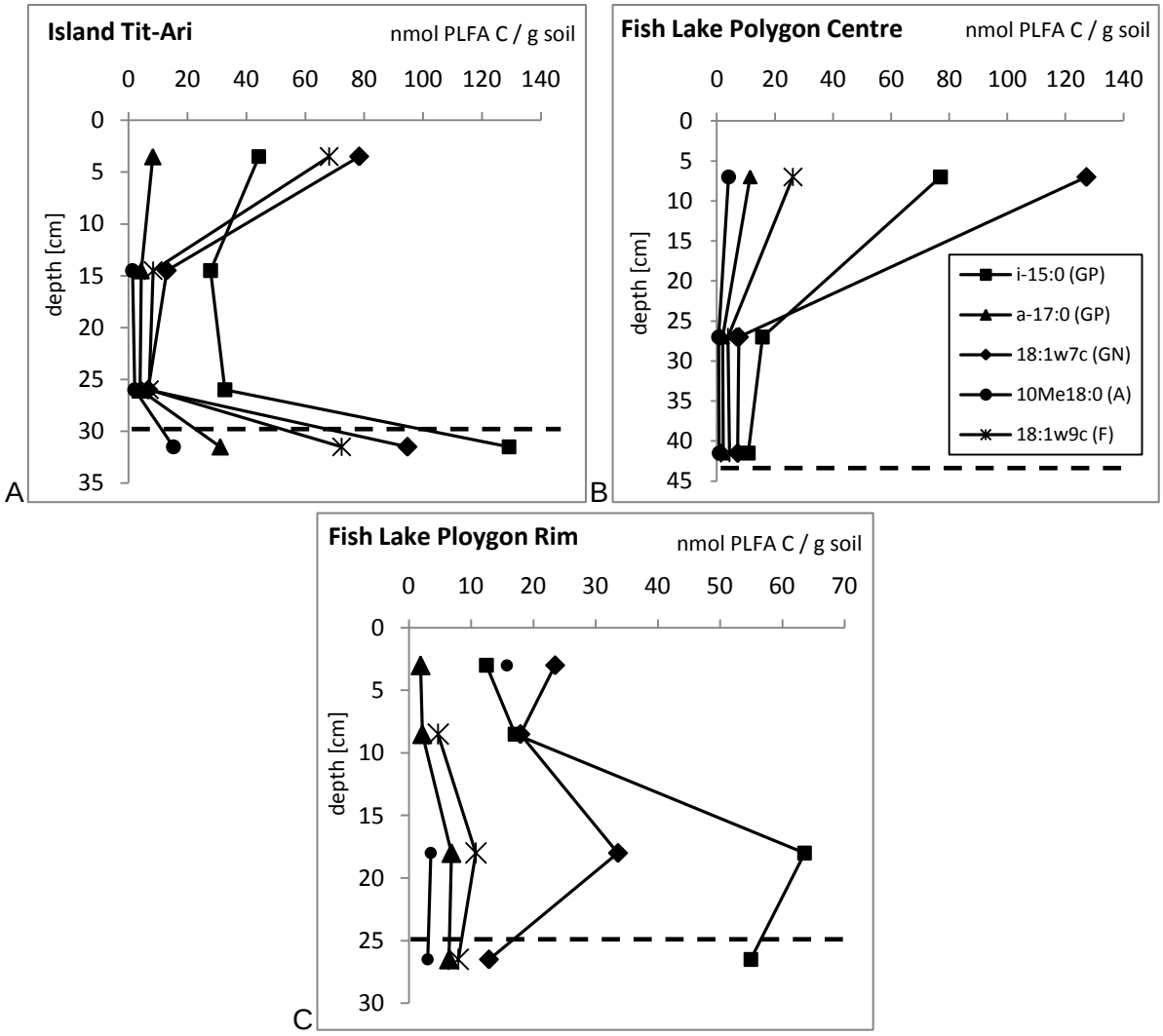

Figure 1: Concentrations of selected PLFAs over depth for three sampling sites in the Lena-Delta. Symbols indicate mid of sampled depth intervals. Doted line (– – –) indicates permafrost base. Letters in brakes name biomarker indication: GP – gram positive bacterial; GN – gram negative bacterial, A – actinomycetes, F – fungal.

At Island Tit-Ari PLFA concentrations decreased with depth within the active layer and increased for the permafrost sample (Fig. 1A). For the Fish Lake Polygon (FLP) Centre (Fig. 1B) concentrations decreased as well with depth. These results indicated a higher microbial activity in the surface soils.

In contrast were the PLFA concentrations of the FLP Rim which increased with depth within the active layer but then decreased for the permafrost sample (Fig. 1C). This result suggests that the organic matter was distributed more equally over the active layer. Furthermore, the decrease of the PLFA concentrations in the permafrost layer compared to the active layer pointed to a lower microbial activity due to a frozen environment as activities decrease with decreasing temperature (Madigan & Martinko 2006).

The stronger decrease of the gram-negative bacterial biomarker (18:1w7c) compared to the gram-positive bacterial biomarker (i-15:0) at all three sites indicated anaerobic conditions closer to the permafrost base. The higher concentrations of the fungal biomarker 18:1w9c at Tit-Ari compared to the other two sites suggested that the organic matter at Tit-Ari contained a higher proportion of more difficult degradable compounds like lignin which fungi can

decompose (Madigan & Martinko 2006). The fact that Island Tit-Ari is the most northern position within the Lena-Delta with tree growth underlined this interpretation.

For all three sampling sites $\delta^{13}C$ values of the selected PLFAs showed no enrichment or depletion over depth ($\delta^{13}C$ range: -42.38‰ to +5.44‰). Thus, no suggestions like Becker-Heidmann & Scharpenseel (1992) made towards more anaerobic (depletion in $\delta^{13}C$ values) or aerobic conditions (enrichment in $\delta^{13}C$ values) could be done from these data. For concluding statement further statistical analysis needs to be done.

2.2) Permafrost profile (Samoylov Cliff)

Samples from the Samoylov Cliff were taken from the side of the cliff after removing the thawed soil to sample only frozen permafrost (Fig. 2A), except for the near- surface sample (55 cm) which was taken from the active layer.

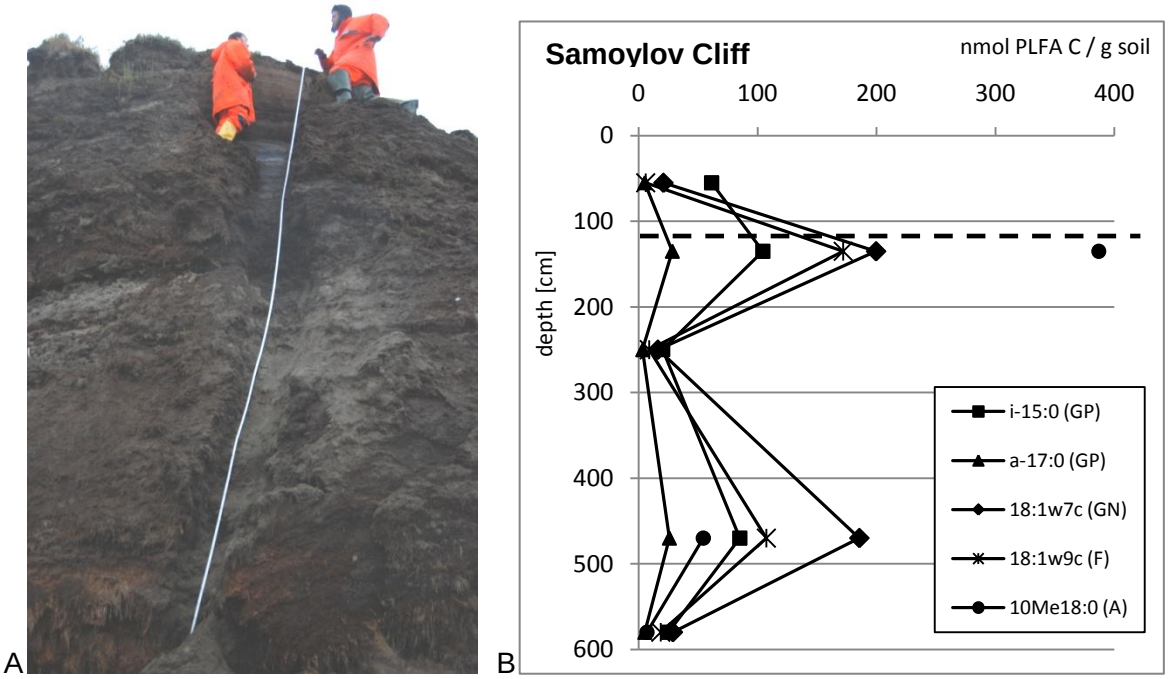

Figure 2: Sampling site “Samoylov Cliff” in the Lena-Delta: A – Photo of sampling (from Gesine Mollenhauer) and B – Concentrations of selected PLFAs over depth. Symbols indicate mid of sampled depth intervals. Doted line (– – –) indicates permafrost base. Letters in brakes name biomarker indication: GP – gram positive bacterial; GN – gram negative bacterial, A – actinomycetes, F – fungal.

PLFA concentrations were lower for the sample of the active layer and for the permafrost sample from 250 cm depth compared to the other three permafrost samples (Fig. 2B). The TOC value at this depth of 250 cm was the highest (9.97 wt-%) measured within the whole profile (1.75 - 8.35 wt-%). Furthermore, the 135 cm and 470 cm depth intervals showed the highest PLFA concentrations but had low TOC values (5.12 and 4.20 wt-%) suggesting a rapid turnover within permafrost that prevents accumulation of organic matter.

2.3) Overall PLFA composition within the Lena Delta

Comparing the overall PLFA composition of all four sites within the Lena-Delta (Fig. 3) samples from Fish Lake Polygon Centre showed a significant different composition than the other three sampling sites (Multivariate Analysis of Variance - MANOVA: $p < 0.05$ for FLP-centre compared to the other three sites). This could be caused by the topographic position

(depression) within the polygon. Due to the seasonal thawing and freezing of the active layer, polygon structures develop in permafrost regions with an elevation difference between elevated rim and depressed centre of several decimetres. At the time of sampling the Centre was covered with water (approximately 3 cm) because the unfrozen water cannot escape from the polygon neither sideward (polygon rim) nor subterraneous (frozen permafrost).

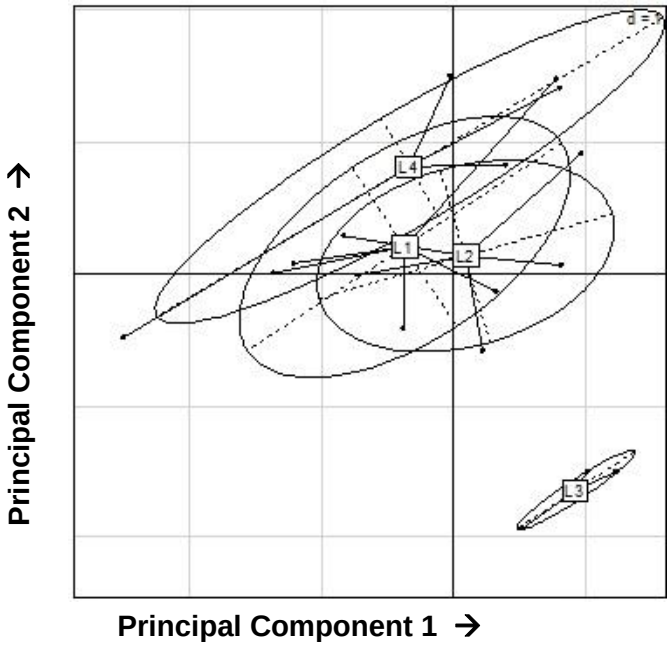

Figure 3: Principal compound analysis of all analysed PLFA concentrations (normalized) of all samples from the Lena-Delta grouped by sampling site (L1 – Samoylov Cliff, L2 – Fish Lake Polygon Rim, L3 – Fish Lake Polygon Centre, L4 – Island Tit-Ari). Points indicate samples. The first two principal components represent 67% of the total variance. The following PLFAs were used: i-14:0, 14:0, i-15:0, a-15:0, 15:0, i-16:0, 2OH12:0, 16:0, 16:1w7t, 10Me16:0, i-17:0, a-17:0, 17:0D9,10, 18:1w9c, 18:1w7c, 18:2w6,9c, 18:3w6,9,12c

3) Study area Svalbard

PLFA concentrations of the active layer profiles from Svalbard decreased with depth (Fig. 3) which suggests a higher microbial activity in the surface soil.

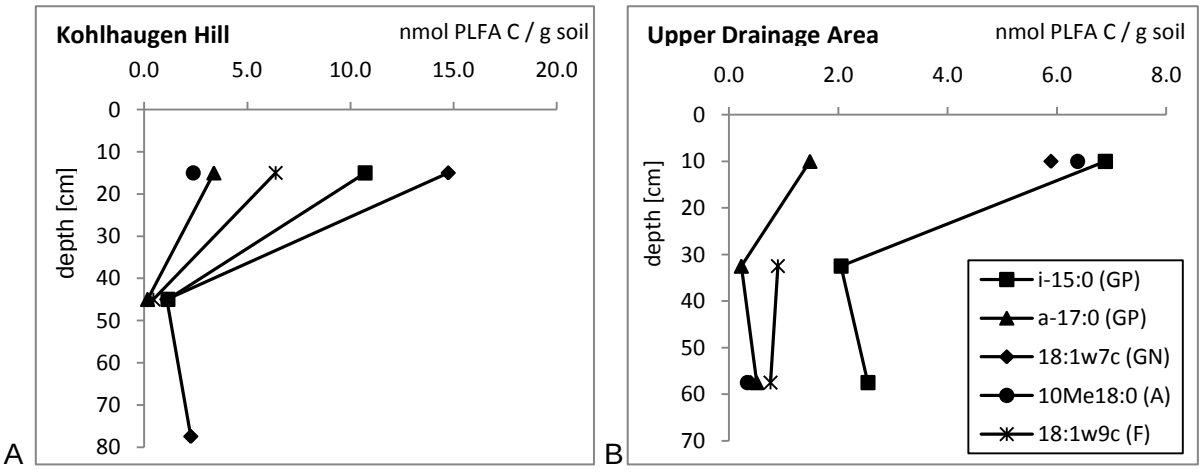

Figure 3: Concentrations of selected PLFAs over depth within the active layer for the two sampling sites in Svalbard. Symbols indicate mid of sampled depth intervals. Letters in brakes name biomarker indication: GP – gram positive bacterial; GN – gram negative bacterial, A – actinomycetes, F – fungal

Decreasing concentrations of the gram-negative bacterial biomarker (18:1w7c), which indicates aerobic conditions (Bossio & Scow 1998), points to more anaerobic environment with increasing depth. This agrees with field observations of more moisture at greater depth. The higher PLFA concentrations for Kohlhaugen Hill were almost twice the concentration for the Upper Drainage Area (UDA) indicating that at the top of the hill microbial activity was higher than in the lower area of this small catchment.

Future collaboration with host institution

The collaboration with Prof. Dr. ir. Pascal Boeckx group at Gent University will be continued in different projects. My work started during this research stay will be continued by ^{14}C analysis of selected PLFAs from Arctic soils. A further collaborative project was started by a diploma student in our group at Cologne University who analysed stable hydrogen and carbon isotopes in soils from Ethiopia which were provided by the working group of Prof. Dr. ir. Pascal Boeckx. This project will also be continued and will include compound-specific ^{14}C analysis of long-chain n-alkanes. Furthermore, it is planed that a PhD student from the host working group at Ghent University comes to Cologne to do ^{14}C-analysis with my working group headed by Prof. Dr. Janet Rethemeyer.

Projected publication to result from the grant

Höfle S, Roobroeck D, Boeckx P, Rethemeyer J: Microbial communities and metabolic activities in permafrost soils (in progress)

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