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Temperature adaptation of microbial communities in different horizons of Siberian permafrost-affected soils from the Lena-Delta

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Abstract

The cell membrane phospholipid (PL) inventory of microbial populations in a Siberian permafrost soil of the Lena Delta were analysed to examine as to how the microbial populations within different horizons of the active layer were adapted to the extreme temperature gradient in this environment. One surface-near and one permafrost-near soil sample were taken from the active layer on Samoylov Island in the southern central Lena Delta (Siberia) and in each case incubated at 4 °C and 28 °C. Subsequently, the phospholipid cell membrane composition of the indigenous microbial populations was qualitatively and quantitatively determined and compared. In both horizons, the incubation at 4 °C is characterized by a shift in the PL inventory to more short chain fatty acids. A significant trend in the proportions of saturated and unsaturated fatty acids, however, was not detected. Both a higher proportion of short chain and of unsaturated fatty acids counterbalances the effect of decreasing cell membrane fluidity with decreasing environmental temperature. Thus, the adaptation of the permafrost microbial populations within the different horizons to varying ambient temperature conditions appears to be mainly regulated by the chain length of the phospholipid fatty acids. Although there is almost no change in the proportions of unsaturated fatty acids between the 4 °C and 28 °C incubation experiments, the permafrost near horizon in general contains more unsaturated fatty acids than the surface near horizon and a higher proportion of short chain fatty acids. This suggests that the lipid inventory of the microbial population nearer to the perennially frozen ground is more adapted to lower temperatures than that of the microbial community from the surface near horizon, which seems to show a higher flexibility toward higher temperature conditions. The permafrost near horizon appears to be dominated by psychrophilic species, while the surface near horizon is characterized by a mesophilic dominated microbial community.

Keywords: Phospholipids, Microorganisms, Temperature adaptation, Permafrost environment, Lena Delta

1. Introduction

Thanks to recent improvements in sensitivity of conventional biogeochemical and microbiological analytical methods and the development of new approaches, microbial life has been detected in environments supposed for long to be hostile (Rothschild and Mancinelli, 2001). The finding of microbial populations in environments like polar- and permafrost areas, hot surface springs and deep sea hydrothermal vents, hypersaline and deep water lakes, mines and oil reservoirs, the deep sea and the deep subsurface of the Earth changed the perspective on the limits of life drastically (e.g. Kashefi and Lovley, 2003; L'Haridon et al., 1995; Liebner et al., 2009; Nisbet and Sleep, 2001; Parkes et al., 2000; Perdersen, 2000; Sharma et al. 2002; Stetter et al., 1990). In addition to pressure, nutrient availability, salinity and alkalinity ambient temperature is one of the most important environmental factors influencing the evolution of microbial life.

Permafrost is a common feature in polar regions covering about 25 % of the northern hemisphere's land surface (Zhang et al., 1999) and can extend to several hundreds of meters depth (e.g. 600 – 800 m in East Siberia). Due to the relatively short summer season in permafrost regions only the surface zone thaws. This so-called active layer is characterized by an extreme temperature regime from about +15 °C to –35 °C, in which a diverse range of microorganisms have been discovered (Kobabe et al., 2004; Liebner et al., 2008). Permafrost soils are a significant source of the climate-relevant trace gas methane (Wagner et al., 2003; Wille et al., 2008). Therefore, permafrost and its microbial communities (e.g. methane producers and consumers) are of specific interest in predicting how the carbon balance of northern ecosystem will respond to climate change. There are only a few studies on microbial diversity (structure and function) in permafrost environments (see Wagner, 2008 and references therein), indicating the need for a comprehensive inventory of these habitats.

Microorganisms are able to inhabit environments with temperature conditions ranging from below 0 °C up to 121 °C (Blöchl et al., 1995; Blöchl et al., 1997, Rothschild and

Mancinelli, 2001; Kashefi and Lovley, 2003, Wagner et al., 2007). Thus, microorganisms must show a high adaptability towards extremely variable ambient temperature conditions. Sinensky (1974) introduced the theory of homeoviscous adaptation. This concept states that microorganisms change their cell membrane lipid composition in order to maintain the cell membrane fluidity and functionality in response to ambient temperature changes. To maintain the membrane fluidity in cold environments microbial cells have to keep their solid-liquid phase transition temperatures (T_c, melting temperature) below the ambient temperature. Thus, the energy supply by ambient temperature is always high enough to keep the cell membranes in a liquid stage. In order to adapt to low temperature conditions, there are several mechanisms known for bacteria and archaea to alter their cell membrane composition accordingly (Russell and Fukunaga, 1990; Suutari and Laakso, 1994). In bacterial cell membranes, largely formed by phospholipid bilayers (phospholipid structures see Appendix 1), commonly two main mechanisms are observed with decreasing environmental temperatures: 1) an increase in the degree of cis-unsaturated phospholipid fatty acids and 2) a higher proportion of short chain phospholipid fatty acids (Suutari and Laakso, 1994). Rather rarely a raise in the proportion of larger and repulsive phospholipid head groups can be observed at lower ambient temperatures (Boggs, 1986). The incorporation of cis double bonds into the phospholipid fatty acid side chains causes a decrease in the melting temperature of the corresponding phospholipid (Russell, 1998; Coolbear et al., 1983), because cis-unsaturation inserts a kink in the otherwise relatively straight fatty acid side chains, preventing a higher compaction of the cell membrane lipids caused by decreasing ambient temperatures (Russell, 1998). A higher proportion of short chain phospholipid fatty acids also leads to a lowering of the cell membrane melting temperature (Suutari and Laakso, 1994), because of the physical effect that the melting temperatures of fatty acyl side chains decreases with decreasing chain length. Additionally, a higher relative proportion of larger and repulsive head groups lowers the melting temperatures again by preventing an increased cell membrane

compaction (Boggs, 1986). Thus, the chemical structure of the different phospholipid groups effects the solid to liquid phase transition temperature of the cell membrane in response to ambient temperature changes (Russell, 1998; Chintalapati et al., 2004).

In the current study, we investigated temperature-dependent changes in the phospholipid cell membrane inventory of two microbial populations from different depth horizons of the active layer from Siberian permafrost environment. Samples of both horizons were incubated at 4 °C and 28 °C and changes in the phospholipid composition were monitored using a high performance liquid chromatography coupled to a mass spectrometer (HPLC-MS) via an electrospray interface (ESI) and collisionally activated dissociation (CAD) experiments (MS-MS).

2. Study area

The study area Samoylov Island is located in the southern central Lena Delta in Northeast Siberia (Fig.1). With 29,000 km^2 (Schneider et al., 2009) the Lena Delta forms the second largest delta of the Arctic Ocean. The whole delta is located within the continuous permafrost zone and the landscape is dominated by ice wedge polygons in different evolutional stages (Schwarmborn et al., 2002). The Lena Delta is affected by a dry continental Arctic climate characterized by a low mean annual air temperature (-14.8 °C) and a summer precipitation of about 200 mm (HMCR, 2004). The winter season lasts eight months, from the end of September to the end of May (T_{avg} = -30 °C, T_{min} = -48 °C) with insufficient light (polar night) and heavy snowstorms up to 140 km h^{-1} (Wein, 1999). The summer period of almost 12 weeks is characterized by moderate temperatures (T_{avg} = 7 °C, T_{max} = 18 °C) and by continuous daylight (polar day).

Samoylov Island (72°22’N, 126°28’E) is located in the southern active part of the Lena Delta in one of the main channels, the Olenyok-channel (Fig. 1). Soil and vegetation characteristics of the investigation area show great variation over small distances owing to the

geomorphological situation of the polygonal tundra (Fiedler et al., 2004; Kutzbach et al., 2004). The soils are characterized by very homogeneously spread soil units. The studied soil of the polygon rim was classified as a *Glacic Aquiturbel* (Soil Survey Staff, 1998). This relatively dry soil is characterized by a soil texture of silty and loamy sand (in this case more from fluvial than aeolian sedimentation), pronounced cryoturbation properties, a distinctly low water level causing oxic conditions in the top soil and a reduced organic matter accumulation (Fiedler et al., 2004). This is reflected by comparatively lower contents of total organic carbon (2.1-3.3 %) and an average of dissolved organic carbon of 8.7 mg l^{-1} (Wagner et al., 2003; Wagner et al., 2005). Low temperature and insufficiency of nitrogen (N_t ranged from 0.2 to 1.4 g kg^{-1}) and phosphor (P_t ranged from 0.47 to 0.63 mg kg^{-1}) led to restricted production of phytomass (Fiedler et al., 2004). The vegetation of the rim is dominated by the dwarf shrub *Dryas punctata* and the mosses *Hylocomium splendens* and *Timmia austriaca*, whereas the adjacent polygon depression is dominated by hydrophytes like various *Carex* species and different moss species (e.g. *Limprichtia revolvens*, *Meesia longiseta*; Kutzbach et al., 2004). During the sampling period in August 2005, the thaw depth of the active layer amounted to about 40 and 55 cm in the rim and in the depression, respectively (Wagner, pers. comm.).

3. Materials and Methods

3.1 Sample material

The investigated soil samples were taken during the expedition LENA 2005 from the active layer of a low-centred ice-wedge polygon (details of patterned ground and cryogenic structure in Wagner (2008)) on Samoylov Island (77°22’N, 126°28’E; Schirrmeister et al., 2007). Samples were taken by an active layer steel corer (Ø = 56 cm, length = 60 cm) from the rim of a polygon. The cores were frozen immediately after sampling and were transported

in a frozen state for further processing to Potsdam, Germany. In the laboratory, core samples were sectioned under sterile conditions and sub-samples for phospholipid analyses were taken from the surface near horizons (11-18 cm) and from the permafrost near horizon (32-38 cm). Phospholipids are relatively unstable due to early diagenetic alteration and therefore are used as marker for viable microbial organisms (White et al., 1979; Harvey et al., 1986; Zink et al., 2003). To avoid potential phospholipids from fresh plant material the upper 11 cm were discarded. The active layer, which is thawed in summer and refreezed in autumn, is in the thawed stage characterized by a distinct temperature gradient from the surface to the permafrost interface ranging from 15 °C to 1 °C.

3.2 Incubation of permafrost soil samples

The two soil horizons (surface and permafrost near horizon, respectively) were homogenised and two aliquots of 5 g of each horizon (three parallels) were placed in different sterile serum flasks. After addition of 5 ml MilliQ-water the flasks were shaken for 20 s. Finally, the soil samples were incubated at 4 °C and 28 °C for 3 weeks. These temperatures were chosen due to the results of microbial activity measurements, which indicated a mesophilic-dominated community in the top soil of the study site, while near the permafrost table a psychrophilic community is dominating (Liebner and Wagner, 2007).

3.3 Analytical procedure

After the incubation, the soil material was extracted with an organic solvent mixture and the organic extracts were separated by column chromatography into fractions of different polarity. The obtained phospholipid fraction was analysed for intact phospholipids using high performance liquid chromatography mass spectrometry (HPLC-MS). For the structural elucidation of individual phospholipids, especially the fatty acyl side chain inventory, collisionally activated dissociation (CAD) MS-MS experiments were performed. Details on

the analytical procedures and instrument conditions applied are described in Zink and Mangelsdorf (2004). For quantification of phospholipids (head group and acyl side chain inventory) obtained from the different soil horizons incubated at different temperatures phospholipids were quantified under consideration of different response factors for individual phospholipid groups during the LC-MS detection process. Details on the relative quantification of phospholipids using HPLC-MS/MS were described in Mangelsdorf et al. (2005).

4. Results

4.1 Qualitative analysis of the phospholipid inventory

The membrane phospholipid (PL) signal of the microbial populations of the different soil horizons investigated consisted of phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylmonomethylethanolamine (PMME) and phosphatidylcholine (PC) esters (Table 1, for structures see Appendix 1). This corresponds to the PL composition of microbial pure cultures from this area also containing PG, PE, PMME and PC esters (data not shown).

The dominating PL in the **surface near** horizon at both incubation temperatures (4 °C and 28 °C) were PCs followed by PMMEs, PGs and finally PEs. Linked saturated and mono unsaturated fatty acids ranged from 14 to 19 carbon atoms for PGs, 14 to 18 for PEs, 13 to 20 for PMMEs and 15 to 20 for PCs (Table 2). While PGs and PEs covered an intermediate range of fatty acids side chain combinations (C_{30}-C_{36} and C_{29} to C_{36}, respectively), PCs comprised the higher end range from C_{31} to C_{38}. PMMEs covered the broadest range of combinations from C_{28} to C_{38}.

In contrast, the **permafrost near** horizon incubations were dominated by PMMEs closely followed by PCs and to a lesser extent by PGs and PEs (Table 1). The fatty acid ranges of the individual PL groups were comparable with those of the surface near horizon. However, C_{13} fatty acids were not detected (Table 3). The range of fatty acid combinations

was also comparable, but diversity of fatty acid combinations within this range was lower. A qualitative difference of the lipid inventory between the 28 °C and 4 °C incubations within each horizon was not detected (Tables 2 and 3).

4.2 Quantitative analysis of the phospholipid inventory

Comparing the 28 °C and 4 °C incubations of the **surface near** horizon it is shown that the relative percentage proportion of PCs decreased by 6.5% from 62.7 to 56.2% (Table 1). This was mainly counterbalanced by an increase of PMMEs from 21.1 to 26.5%, while PGs and PEs remained relatively constant. A comparable picture can be observed in the 28 °C and 4 °C incubations of the **permafrost near** horizon. PCs decreased by 6.4% from 40.7% to 34.3% and PMMEs increased from 43.1% to 50%, while PGs and PEs remained constant with comparable percentages as in the surface near horizon (Table 1). Thus, both horizons showed the same shifts during the temperature experiments. The main difference was that the surface near horizon was dominated by PCs and the permafrost near horizon by PMMEs.

The quantitative distribution of the fatty acyl side chain inventory of the **surface near** microbial population incubated at 4 °C and 28 °C is shown in Figures 2a and 2b. For better visualization of the changes in the fatty acyl side chain inventory between both incubation experiments a difference diagram was created by subtracting the fatty acid distribution at 28 °C from that at 4 °C (Fig. 2c). It can be recognized that the fatty acid inventory of the 4 °C incubation was composed of less long chain fatty acids and more short chain fatty acids. A clear shift from 37.9% (28 °C incubation) to 45.1% (4 °C incubation) of 7.2% to more short chain fatty acids is discernable, whereas the percentage proportions of saturated and unsaturated fatty acids did not change significantly (Table 4).

The quantitative distribution of the fatty acyl side chain inventory of the **permafrost near** microbial population incubated at 4 °C and 28 °C is shown in Figures 3a and 3b. The same trend to more short chain fatty acids in the 4°C incubation can be observed. This shift

was somewhat larger (10.3%) than in the surface near horizon (Table 4). The relative proportions of saturated and unsaturated fatty acids remained also relatively constant during the incubation experiments.

However, with 73.2% and 72.2% the **permafrost near** microbial population contained on average about 5.7% more unsaturated fatty acids than the **surface near** population. With 50.1% and 60.4% it also contained about 12 to 15% more short chain fatty acids compared to the population of the surface near horizon with 37.9% and 45.1% (Table 4).

A deeper insight into the membrane alteration during the temperature experiments for both horizons is provided by Figures 4 and 5. In these figures, the relative proportions of the individual PL groups on the total fatty acid side chain inventory (Figs. 2 and 3) are presented. Both figures revealed a minor influence of the PG and PE signals on the total fatty acid distribution, although a trend towards more short chain fatty acids was discernable in the PG signal of the surface near horizon (Fig. 4). Main changes derived from the alteration of the PC and PMME lipid inventory as already suggested from Table 1. Long chain fatty acids were preferentially lost during the decrease of PCs between the 28 °C and 4 °C incubation in both horizons. The increase of short chain fatty acids in the 4 °C incubations in both horizons was predominantly linked to the increase of PMMEs.

5. Discussion

The seasonal unfrozen part of the permafrost landscape (active layer, approximately 0.5 m soil depth at the study site) is subjected to freezing and thawing cycles during the year with a large temperature gradient between 15 °C and 1 °C along their depth profile in the thawed stage. This temperature gradient is one of the main environmental factors influencing microorganisms in permafrost-affected soils. The phospholipid inventory used for the characterization of the adaptation of microorganisms to the low *in situ* temperature of their extreme habitat showed significant differences in the relative phospholipid composition

between active layer top and bottom on one hand and between the 4 °C and 28 °C incubation experiments on the other hand.

5.1 Changes in the phospholipid head group composition

The PL signal of the **surface near** microbial population is dominated by PCs, while that of the **permafrost near** microbial community is dominated by PMMEs. The relative proportion of PGs and PEs is quite similar in both horizons and seem to show no significant temperature dependent variability. In contrast, both horizons reveal an increase of PMMEs in the 4 °C incubation. Thus, the observed trend in both temperature incubation experiments together with the dominance of PMMEs in the indigenous microbial population nearer to the perennially frozen ground suggests a shift to more PMMEs at lower temperature conditions in this permafrost environment.

It is suggested that larger head groups like those of the PGs and PCs cause a greater disturbance of the cell membrane compaction than the smaller PE head group. Additionally, PEs are able to interact intermolecularly by way of hydrogen-bonding leading to a higher membrane compaction (Boggs, 1986; Russell, 1989; Fang et al., 2000). The phase transition temperatures of PG (16:0/16:0) and PC (16:0/16:0) being both ca. 41 °C are about 20 °C lower than that of PE (16:0/16:0) with a value of ca. 63 °C (Cullis et al., 1996). The corresponding PMME, the mono-*N*-methylated congener of PE, shows with ca. 58 °C a phase transition temperature between those of the PC (tri-*N*-methylated PE) and PE (Casal and Mantsch, 1983). Thus, membrane phase transition temperature decreases in a series from PE>PMME> PC=PG.

Considering this the observed shift to more PMMEs at lower temperature conditions seems to contradict the concept of incorporating more of larger and repulsive head groups to lower the phase transition temperature. However, according to the phospholipid side chain analyses (Table 2 and 3) it can be deduced that PCs only cover the higher end of fatty acid

combinations (C_{31}-C_{38}) with a maximum at C_{18} (Figs. 4 and 5). In contrast, PMMEs cover the broadest range of combinations (C_{28}-C_{38}) including many short chain fatty acid combinations and a maximum at C_{16} (Figs. 4 and 5). The incorporation of more short chain fatty acid combinations is an important adaptation in low temperature environments to keep the phase transition temperatures below the ambient temperature (see below). Thus, these data suggest that the relative proportions of head groups are not determined by their effect directly on the cell membrane fluidity but rather by the spectrum of fatty acids linked to the different PL groups.

5.2 Ratio of unsaturated to saturated phospholipid fatty acids

A higher proportion of *cis*-unsaturated fatty acids also leads to a decrease of the phase transition temperature of cell membranes (Cossins, 1983), because the bended configuration of the cis-unsaturated phospholipid fatty acid side chain prevents a closer cell membrane packing. In fact, cis-unsaturation causes a pronounced decrease of the phase transition temperature (PC (18:0/18:0) = 55.8 °C and PC (18:0/18:1) = 6.3 °C; from Russell, 1989).

In both horizons, saturated and mono-unsaturated fatty acids were detected, which is common for bacteria (Russell and Fukunaga, 1990). Poly-unsaturated fatty acids as often observed in response to higher ambient pressure (DeLong and Yayanos, 1985; Yano et al., 1998; Nogi and Kato, 1999) were not detected. High pressure and low temperature seem to cause comparable compositional membrane lipid adaptations. The incorporation of one double bond has the largest effect on the solid-liquid phase transition temperature and more than 2 double bonds appear to have the opposing effect by increasing the rigidity of the fatty acyl chains (Coolbear et al., 1983; Suutari and Laakso, 1994). The PL signals of the surface near as well as of the permafrost near horizon reveal both a high content of mono-unsaturated fatty acids (on average 67% and 72.7%, respectively) indicating a high adaptation to the low temperature conditions in permafrost areas. On average, the **permafrost near** horizon reveals

5.7% more unsaturated fatty acids than the **surface near** horizon. Thus, the microbial population nearer to the perennially frozen ground seems to show a higher adaptation to cooler ambient conditions.

Comparing the temperature experiments there is no significant change in the ratio between saturated and unsaturated fatty acids. Although figures 2 and 3 show an increase of short chain unsaturated fatty acids, this is concomitantly outweighed by the loss of long chain unsaturated fatty acids. Thus, surprisingly the microbial communities within the different depth horizons seem not to alter their degree of unsaturation significantly as an adaptation to lower ambient temperatures.

5.3 Ratio of short chain to long chain phospholipid fatty acids

A shortening of the chain length of the phospholipid fatty acids also causes a decrease in the melting temperatures of the PLs (Russell, 1989). For instance, the phase transition temperature of the PC (16:0/14:0) is with 27 °C about 14 °C lower than that of the PC (16:0/16:0) with 41 °C and about 28 °C lower than that of the PC (18:0/18:0) with 55 °C (Keough and Davis, 1979; Russell, 1989; Cullis et al., 1996). Thus, the shift to shorter fatty acid forms another microbial adaptation mechanism to maintain the cell membrane fluidity.

The **permafrost near** horizon shows a 12 to 15% higher proportion of short chain fatty acids than the **surface near** horizon, indicating again a higher adaptation of the **permafrost near** microbial population to the lower temperatures adjacent to the perennially frozen ground. The 4 °C temperature incubation experiments reveal for both horizons a significant increase of short chain fatty acids associated to the alteration from less PCs to more PMMEs (Figs. 4 and 5), whereas this trend is somewhat larger within the **permafrost near** microbial community (Table 4). This suggests that within the investigated horizons the maintenance of the cell membrane fluidity at decreasing ambient temperature conditions is mainly regulated by the chain length of the phospholipid fatty acids.

5.4 Origin for the compositional changes of the phospholipid inventory

These results raise the question where the observed compositional changes of the phospholipid cell membrane inventories originate from: a direct restructuring of the cell membranes of the indigenous microbial community during the 3 weeks of incubation or a relative preferential increase (dominance) of specific microbes especially adapted to the different incubation temperature conditions.

Liebner and Wagner (2007) reported that the permafrost soil samples contain overall a mixture of mesophilic and psychrophilic microbes, whereas the top soil is dominated by mesophilic and the permafrost near part by psychrophilic microorganisms. A trend of the overall composition of the microbiota along an *in situ* temperature gradient in permafrost soils of the active layer is supported by a study of Liebner et al. (2008). They showed a shift of the bacterial community along the soil profile by clone library analysis. Particularly, the *Bacteroidetes-Chlorobi* phylum drastically increased with increasing soil depth. This group is characterized by psychrophilic species (Shivaji et al., 1991). This suggests that the general difference in the phospholipid distribution between the permafrost near and surface near microbial communities (more short chain and unsaturated phospholipid fatty acid side chains in the permafrost near population) are caused by different proportions of mesophilic (higher in surface near horizon) and psychrophilic (higher in permafrost near horizon) microbial species in the two horizons investigated (Table 4).

Within the different horizons the compositional changes during the 4°C and 28°C incubation experiments might also partly derive from a relative increase of mesophilic microorganisms in the 28 °C incubation and psychrophilic species in the 4 °C incubation. However, the fact that only a shift to more short chain phospholipid fatty acid side chains can be observed during the temperature experiments and not also to more unsaturated fatty acid side chains (Table 4) points to an additional adaptive restructuring of the cell membrane lipid

composition within the mesophilic and psychrophilic species in response to the ambient temperature conditions.

6. Conclusions

The aim of the current study was to examine as to how the microbial populations within the different horizons of the active layer were adapted to the extreme variable temperature regime of the permafrost-affected soils. Therefore, two soil samples were taken from the active layer on Samoylov Island in the southern central Lena Delta, Siberia and aliquots of each sample were incubated under 4 °C and 28 °C.

The comparison of the phospholipid fatty acid (PLFA) distribution of the incubation experiments within the **surface near** and the **permafrost near** horizons shows no significant change in the proportion of unsaturated fatty acids under cold temperature conditions (4 °C) in comparison to higher temperatures (28 °C). In contrast to this finding the **surface near** as well as the **permafrost near** microbial communities reveal both a distinct relative increase of short chain fatty acids of 7.3 and 10.3%, respectively, after an incubation at 4 °C. As fatty acids with shorter chain length lower the solid-liquid phase transition temperatures of the corresponding phospholipid, these results indicate that the adaptation towards lower ambient temperatures is mainly regulated by a higher proportion of short chain phospholipid fatty acid side chains.

In addition to this significant chain length adaptation, the PLFA proportions of the microbial population of the active layer differ between the surface near and permafrost near horizon. The microbial community closer to the perennially frozen ground shows in general a higher relative proportion of unsaturated and shorter chain fatty acids. This indicates a stronger adaptation to cold *in situ* temperature (constantly < 2 °C), whereas the **surface near** population appears to reveal a higher flexibility towards warmer temperature conditions.

It is suggested that the general compositional changes in the phospholipid inventories between both horizons derived from a relative dominance of mesophilic species in the surface near and psychrophilic species in the permafrost near horizon. However, the 4°C and 28°C incubation experiments also suggest that an adaptive restructuring of the cell membrane composition within the mesophilic and psychrophilic communities in response to environmental temperature conditions is additionally responsible for the compositional changes in the phospholipid membrane inventory.

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Figure Caption:

Figure 1: Location of the Lena Delta in NE Siberia. The subset map shows the distribution of the three main geomorphologic terraces in the delta (based on Schwamborn et al., 2002) with the location of the study area Samoylov Island (black circle).

Figure 2: Phospholipid fatty acid (PLFA) side chain distribution of the microbial community from the surface near horizon incubated at 4 °C (2a) and 28 °C (2b). Difference diagram (2c) of the fatty acid distribution incubated at 4 °C and 28 °C. Unsat. and sat. FA = unsaturated and saturated fatty acids. x:y = carbon number:number of double bonds.

Figure 3: Phospholipid fatty acid (PLFA) side chain distribution of the microbial community from the permafrost near horizon incubated at 4 °C (2a) and 28 °C (2b). Difference diagram (2c) of the fatty acid distribution incubated at 4 °C and 28 °C. Unsat. and sat. FA = unsaturated and saturated fatty acids. x:y = carbon number:number of double bonds.

Figure 4: Fatty acid distribution pattern of phosphatidylglycerols (PGs), phosphatidylethanolamines (PEs), phosphatidylmonomethylethanolamines (PMMEs) and phosphatidylcholines (PCs) in their percentage proportion of the total fatty acid distribution of the microbial population from the surface near horizon incubated at 4 °C and 28 °C. Difference diagram of the fatty acid distribution of individual phospholipid groups in their percentage proportion incubated at 4 °C and 28 °C. Unsat. and sat. FA = unsaturated and saturated fatty acids. x:y = carbon number:number of double bonds.

Figure 5: Fatty acid distribution pattern of phosphatidylglycerols (PGs), phosphatidylethanolamines (PEs), phosphatidylmonomethylethanolamines (PMMEs) and phosphatidylcholines (PCs) in their percentage proportion of the total fatty acid distribution of the microbial population from the permafrost near horizon incubated at 4 °C and 28 °C. Difference diagram of the fatty acid distribution of individual phospholipid groups in their percentage proportion incubated at 4 °C and 28 °C. Unsat. and sat. FA = unsaturated and saturated fatty acids. x:y = carbon number:number of double bonds.

Table captions:

Table 1: Percentage proportions of different phospholipid groups of the total phospholipid signal of the microbial population in the surface near and permafrost near soil horizons incubated at 4 °C and 28 °C.

Table 2: Fatty acid combinations linked to the different phospholipid groups of the total phospholipid signal of the microbial population in the surface near soil horizon incubated at 4 °C and 28 °C.

Table 3: Fatty acid combinations linked to the different phospholipid groups of the total phospholipid signal of the microbial population in the permafrost near soil horizon incubated at 4 °C and 28 °C.

Table 4: Percentage proportions of unsaturated and saturated phospholipid fatty acids (PLFA) and short chain (C_{13}-C_{16}) and long chain (C_{17}-C_{20}) PLFA of the microbial population in the surface near and permafrost near soil horizons incubated at 4 °C and 28 °C.

Appendix 1: Molecular structure of the different phospholipid groups detected in the soil sediments of the current study: a) PG = phosphatidylglycerol; b) PE = phosphatidylethanol-amine; c) PMME = phosphatidylmonomethylethanolamine; d) PC = phosphatidylcholine. R_1 and R_2 = saturated or unsaturated alkyl side chains with various numbers of carbon atoms.

Table 1:

Phospholipid group	Surface near horizon (11-18 cm)		Permafrost near horizon (25-32 cm)	
	28 °C (%)	4 °C (%)	28 °C (%)	4 °C (%)
PG	10.2	11.1	9.9	9.7
PE	5.9	6.2	6.2	6.1
PMME	21.1	26.5	43.1	50.0
PC	62.7	56.2	40.7	34.3

PG = phosphatidylglycerol; PE = phosphatidylethanolamine; PMME = phosphatidylmonomethylethanolamine;

PC = phosphatidylcholine.

Tabelle 2:

Number of carbon atoms and double bonds of linked FA	Linked fatty acid combinations	PG 28 °C (4 °C)	PE 28 °C (4 °C)	PMME 28 °C (4 °C)	PC 28 °C (4 °C)
$C_{28:1}$	13:0/15:1	-	-	X / (X)	-
	14:0/14:1	-	-	X / (X)	-
	15:0/13:1	-	-	X / (X)	-
$C_{29:1}$	14:0/15:1	-	X / (X)	X / (X)	-
	15:0/14:1	-	X / (X)	-	-
$C_{29:0}$	15:0/14:0	-	-	X / (X)	-
$C_{30:2}$	16:1/14:1	X / (X)	-	-	-
$C_{30:1}$	14:0/16:1	X / (X)	X / (X)	X / (X)	-
	15:0/15:1	X / (X)	-	X / (X)	-
	16:0/14:1	X / (X)	-	-	-
$C_{30:0}$	15:0/15:0	X / (X)	-	X / (X)	-
	16:0/14:0	X / (X)	-	-	-
$C_{31:2}$	16:1/15:1	X / (X)	-	X / (X)	-
$C_{31:1}$	15:0/16.1	X / (X)	X / (X)	X / (X)	X / (X)
	16:0/15:1	X / (X)	-	X / (X)	-
$C_{31:0}$	16:0/15:0	X / (X)	-	X / (X)	X / (X)
$C_{32:2}$	16:1/16:1	X / (X)	X / (X)	X / (X)	X / (X)
	17:1/15:1	-	X / (X)	X / (X)	X / (X)
$C_{32:1}$	16:0/16:1	X / (X)	X / (X)	X / (X)	X / (X)
	15:0/17:1	-	X / (X)	X / (X)	-
	17:0/15:1	-	-	X / (X)	-
$C_{32:0}$	16:0/16:0	X / (X)	X / (X)	-	-
	17:0/15:0	X / (X)	X / (X)	-	X / (X)
$C_{33:2}$	16:1/17:1	X / (X)	-	X / (X)	X / (X)
$C_{33:1}$	16:0/17:1	X / (X)	X / (X)	X / (X)	X / (X)
	17:0/16:1	-	X / (X)	X / (X)	X / (X)
$C_{33:0}$	16:0/17:0	X / (X)	-	X / (X)	X / (X)
$C_{34:2}$	18:1/16:1	X / (X)	X / (X)	X / (X)	X / (X)
	17:1/17:1	-	X / (X)	X / (X)	-
$C_{34:1}$	16:0/18:1	X / (X)	X / (X)	X / (X)	X / (X)
	18:0/16:1	X / (X)	X / (X)	-	X / (X)
	17:0/17:1	-	X / (X)	X / (X)	-
$C_{34:0}$	18:0/16:0	X / (X)	X / (X)	X / (X)	-
$C_{35:2}$	18:1/17.1	X / (X)	-	X / (X)	X / (X)
$C_{35:1}$	17:0/18:1	-	X / (X)	X / (X)	X / (X)
	18:0/17:1	X / (X)		X / (X)	X / (X)
$C_{36:2}$	18:1/18:1	X / (X)	X / (X)	X / (X)	X / (X)
	19:1/17:1	X / (X)	-	X / (X)	X / (X)
$C_{36:1}$	17:0/19:1	X / (X)	-	X / (X)	X / (X)
	18:0/18:1	X / (X)	-	X / (X)	X / (X)
$C_{37:2}$	19:1/18:1	-	-	X / (X)	X / (X)
$C_{37:1}$	19:0/18:1	-	-	X / (X)	X / (X)
	18:0/19:1	-	-	-	X / (X)
$C_{38:2}$	19:1/19:1	-	-	X / (X)	X / (X)
	20:1/18:1	-	-	X / (X)	X / (X)
$C_{38:1}$	18:0/20:1	-	-	X / (X)	-

	19:0/19:1	-	-	X / (X)	-

PG = phosphatidylglycerol; PE = phosphatidylethanolamine; PMME = phosphatidylmonomethylethanolamine;

PC = phosphatidylcholine. FA = fatty acids.

Table 3:

Number of carbon atoms and double bonds of linked FA	Linked fatty acid combinations	PG 28 °C (4 °C)	PE 28 °C (4 °C)	PMME 28 °C (4 °C)	PC 28 °C (4 °C)
$C_{28:1}$	14:0/14:1	-	-	X / (X)	-
$C_{29:1}$	14:0/15:1	-	X / (X)	X / (X)	-
	15:0/14:1	-	X / (X)	-	-
$C_{29:0}$	15:0/14:0	-	-	X / (X)	-
$C_{30:1}$	14:0/16:1	X / (X)	X / (X)	X / (X)	-
	15:0/15:1	X / (X)	-	X / (X)	-
	16:0/14:1	X / (X)	-	-	-
$C_{30:0}$	15:0/15:0	X / (X)	X / (X)	X / (X)	-
	16:0/14:0	X / (X)	X / (X)	-	-
$C_{31:2}$	16:1/15:1	-	-	X / (X)	-
$C_{31:1}$	15:0/16.1	-	X / (X)	X / (X)	-
	16:0/15:1	-	-	X / (X)	-
$C_{32:2}$	16:1/16:1	X / (X)	X / (X)	X / (X)	X / (X)
	17:1/15:1	-	X / (X)	X / (X)	X / (X)
$C_{32:1}$	16:0/16:1	X / (X)	X / (X)	X / (X)	X / (X)
	15:0/17:1	-	X / (X)	X / (X)	-
	17:0/15:1	-	-	X / (X)	-
$C_{32:0}$	16:0/16:0	X / (X)	X / (X)	-	-
	17:0/15:0	X / (X)	X / (X)	-	-
$C_{33:2}$	16:1/17:1	-	-	X / (X)	-
$C_{33:1}$	16:0/17:1	X / (X)	X / (X)	-	X / (X)
	17:0/16:1	-	X / (X)	-	X / (X)
$C_{33:0}$	16:0/17:0	X / (X)	-	-	-
$C_{34:2}$	18:1/16:1	X / (X)	X / (X)	X / (X)	X / (X)
	17:1/17:1	-	X / (X)	X / (X)	-
$C_{34:1}$	16:0/18:1	-	X / (X)	X / (X)	X / (X)
	18:0/16:1	-	X / (X)	-	X / (X)
	17:0/17:1	-	X / (X)	X / (X)	-
$C_{35:2}$	18:1/17.1	-	-	X / (X)	X / (X)
$C_{36:2}$	18:1/18:1	X / (X)	X / (X)	X / (X)	X / (X)
	19:1/17:1	X / (X)	-	X / (X)	X / (X)
$C_{37:2}$	19:1/18:1	-	-	-	X / (X)
$C_{37:1}$	19:0/18:1	-	-	-	X / (X)
	18:0/19:1	-	-	-	X / (X)
$C_{38:2}$	19:1/19:1	-	-	X / (X)	X / (X)
	20:1/18:1	-	-	X / (X)	X / (X)

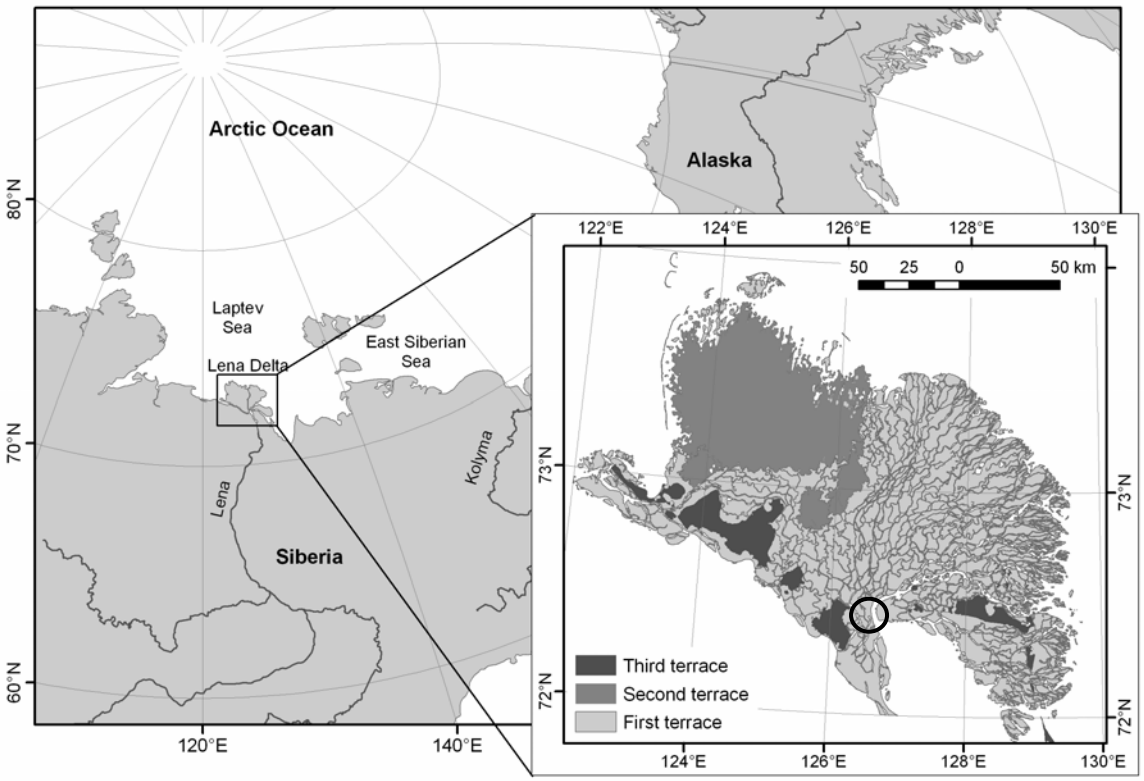
PG = phosphatidylglycerol; PE = phosphatidylethanolamine; PMME = phosphatidylmonomethylethanolamine;

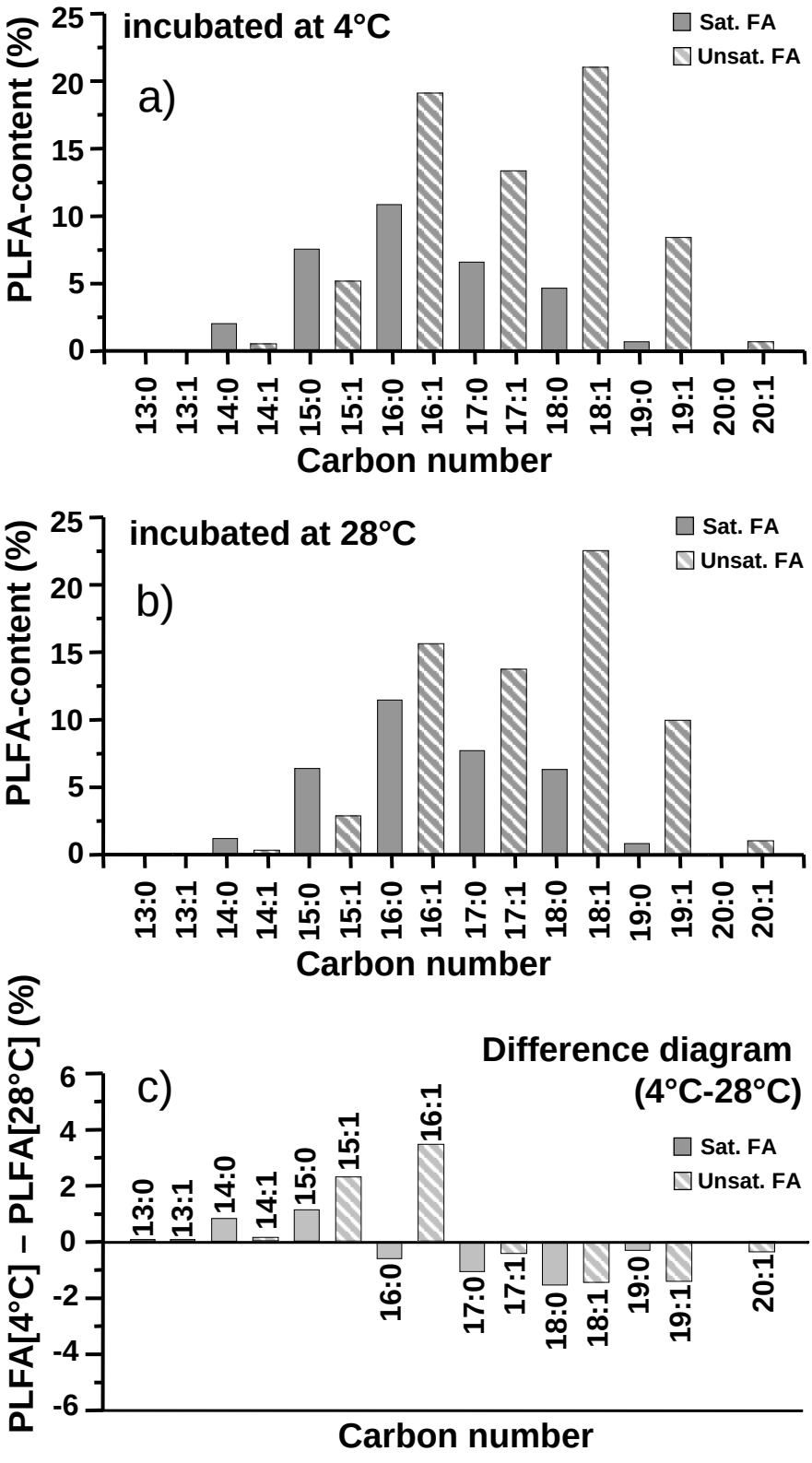
PC = phosphatidylcholine. FA = fatty acids.

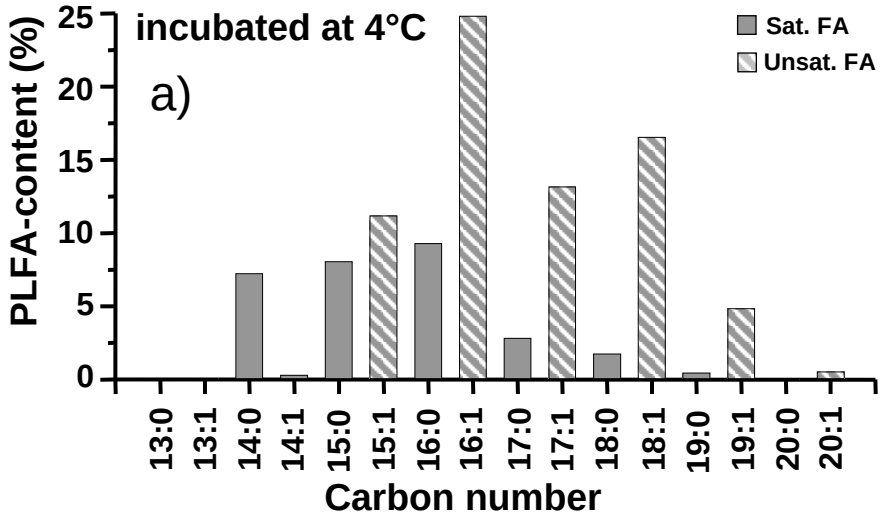
Table 4:

Parameter	Surface near horizon (11-18 cm)		Permafrost near horizon (25-32 cm)	
	28 °C (%)	4 °C (%)	28 °C (%)	4 °C (%)
Unsat. FA	66.1	67.9	73.2	72.2
Sat. FA	33.9	32.1	26.8	27.8
C_{17}-C_{20} FA (long)	62.1	54.9	49.9	39.6
C_{13}-C_{16} FA (short)	37.9	45.1	50.1	60.4

Unsat. FA = unsaturated fatty acids; Sat. FA = saturated fatty acids.


Arctic Ocean
Alaska
Laptev Sea
East Siberian Sea
Lena Delta
Lena
Kolyma
Siberia
80°N
70°N
60°N
120°E
140°E
122°E
124°E
126°E
128°E
130°E
73°N
72°N
50 25 0 50 km
Third terrace
Second terrace
First terrace


incubated at 4°C
a)
Sat. FA
Unsat. FA
PLFA-content (%)
25
20
15
10
5
0
13:0
13:1
14:0
14:1
15:0
15:1
16:0
16:1
17:0
17:1
18:0
18:1
19:0
19:1
20:0
20:1
Carbon number
incubated at 28°C
b)
Sat. FA
Unsat. FA
PLFA-content (%)
Carbon number
Difference diagram
(4°C-28°C)
c)
Sat. FA
Unsat. FA
PLFA[4°C] – PLFA[28°C] (%)
6
4
2
0
-2
-4
-6
Carbon number


incubated at 4°C
a)
Sat. FA
Unsat. FA
PLFA-content (%)
25
20
15
10
5
0
13:0
13:1
14:0
14:1
15:0
15:1
16:0
16:1
17:0
17:1
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18:1
19:0
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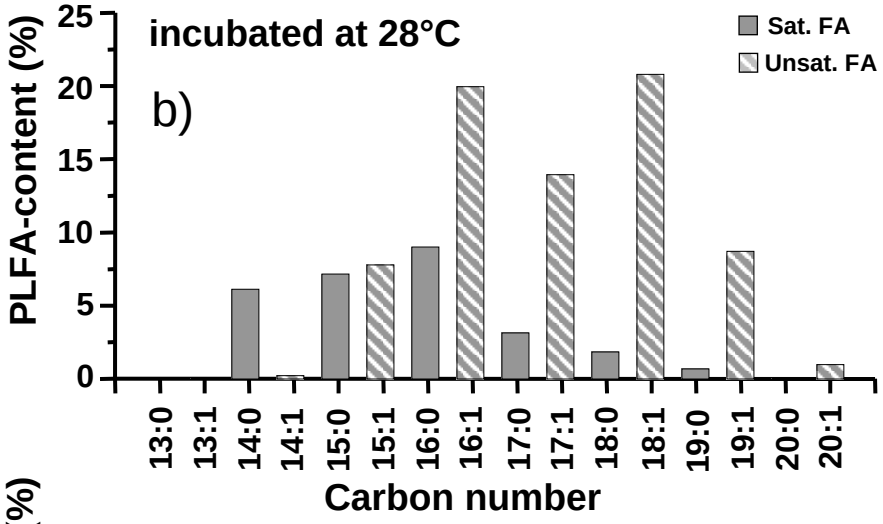
20:0
20:1
Carbon number


incubated at 28°C
b)
Sat. FA
Unsat. FA
PLFA-content (%)
25
20
15
10
5
0
13:0
13:1
14:0
14:1
15:0
15:1
16:0
16:1
17:0
17:1
18:0
18:1
19:0
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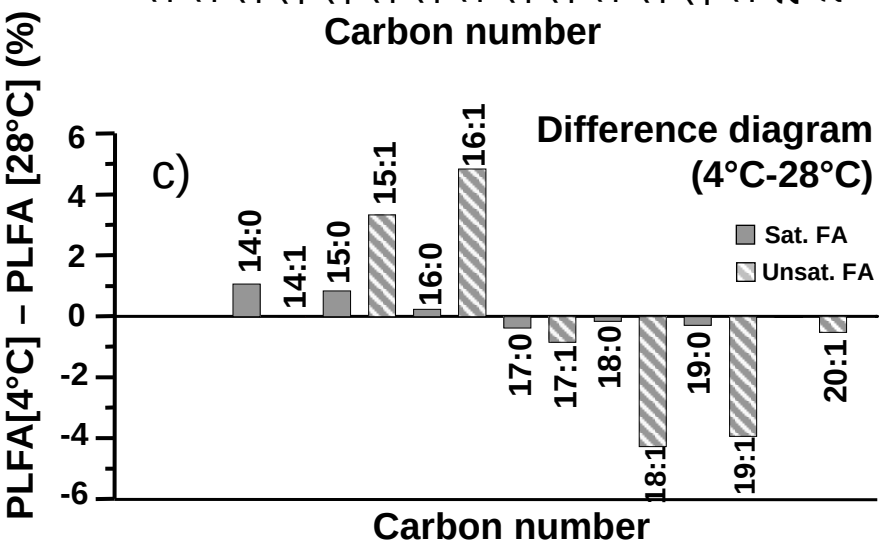
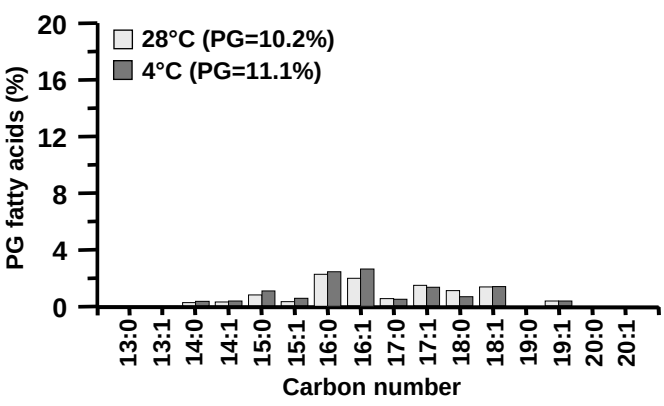
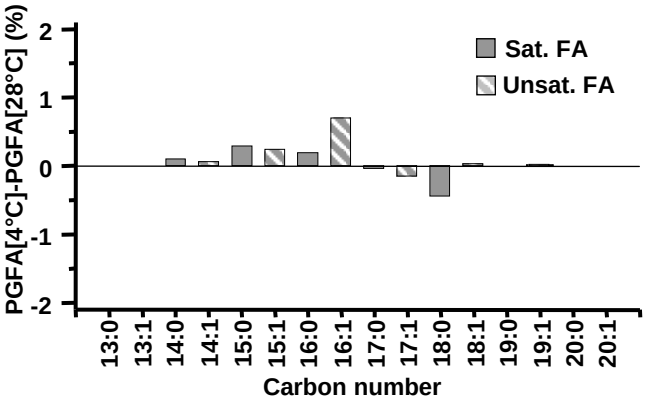
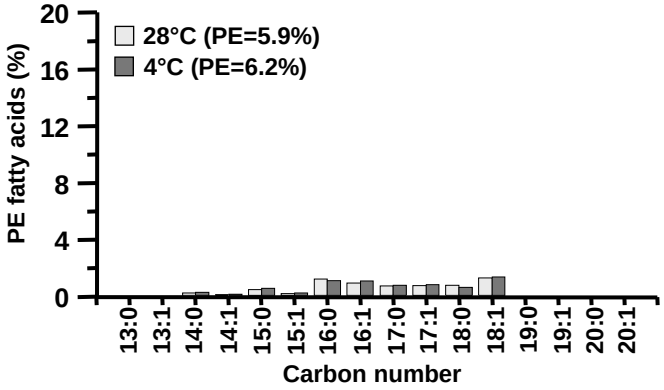
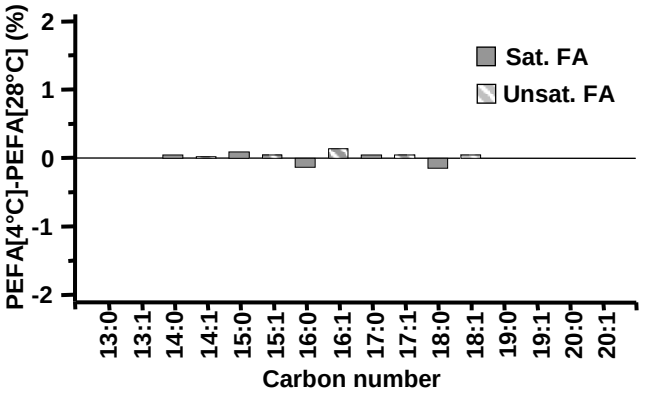
20:0
20:1
Carbon number

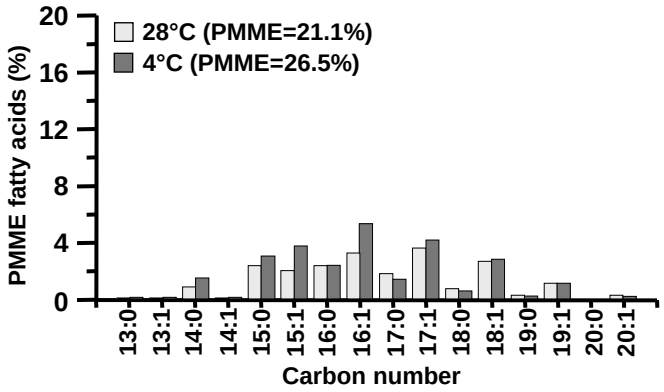

c)
Difference diagram
(4°C-28°C)
Sat. FA
Unsat. FA
PLFA[4°C] – PLFA [28°C] (%)
6
4
2
0
-2
-4
-6
14:0
14:1
15:0
15:1
16:0
16:1
17:0
17:1
18:0
18:1
19:0
19:1
20:1
Carbon number

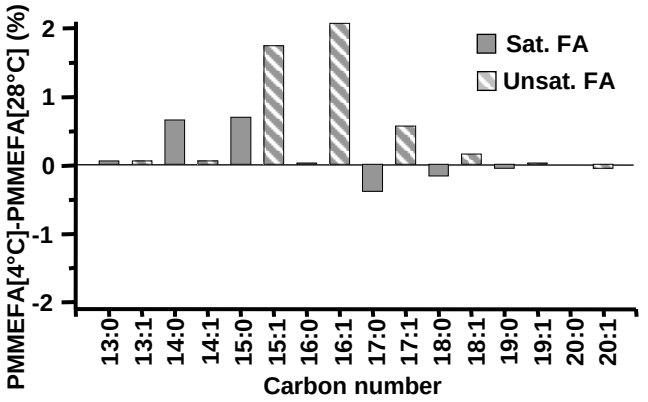

28°C (PG=10.2%)
4°C (PG=11.1%)
PG fatty acids (%)
0
4
8
12
16
20
13:0
13:1
14:0
14:1
15:0
15:1
16:0
16:1
17:0
17:1
18:0
18:1
19:0
19:1
20:0
20:1
Carbon number

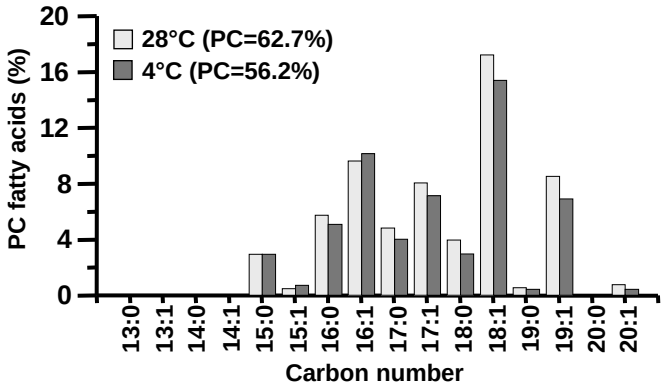

Sat. FA
Unsat. FA
PGFA[4°C]-PGFA[28°C] (%)
-2
-1
0
1
2
13:0
13:1
14:0
14:1
15:0
15:1
16:0
16:1
17:0
17:1
18:0
18:1
19:0
19:1
20:0
20:1
Carbon number

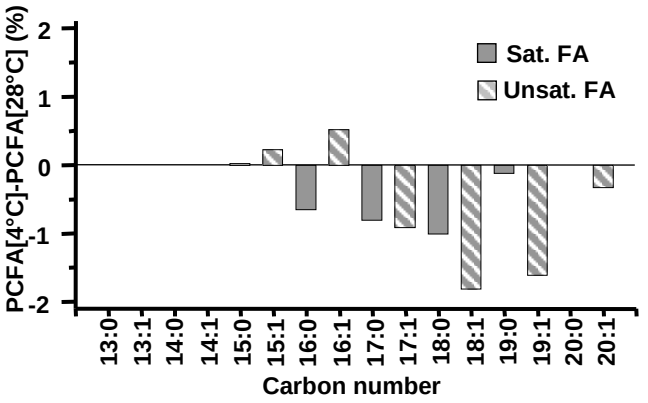

28°C (PE=5.9%)
4°C (PE=6.2%)
PE fatty acids (%)
0
4
8
12
16
20
13:0
13:1
14:0
14:1
15:0
15:1
16:0
16:1
17:0
17:1
18:0
18:1
19:0
19:1
20:0
20:1
Carbon number

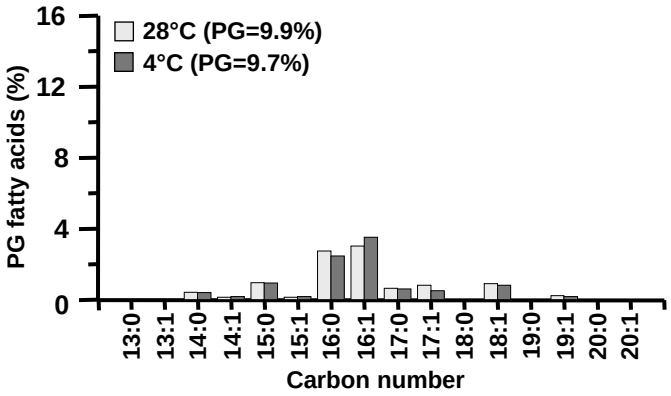

Sat. FA
Unsat. FA
PEFA[4°C]-PEFA[28°C] (%)
-2
-1
0
1
2
13:0
13:1
14:0
14:1
15:0
15:1
16:0
16:1
17:0
17:1
18:0
18:1
19:0
19:1
20:0
20:1
Carbon number

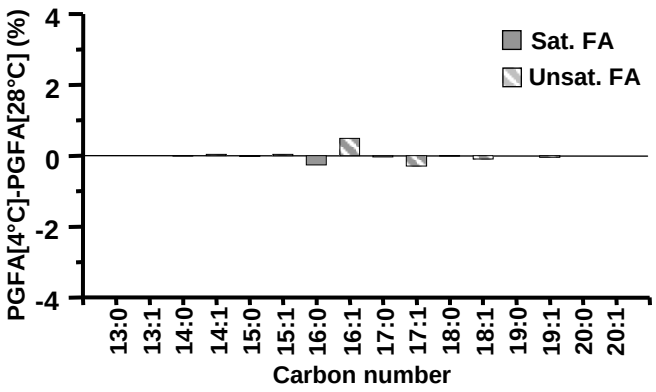

28°C (PMME=21.1%)
4°C (PMME=26.5%)
PMME fatty acids (%)
0
4
8
12
16
20
13:0
13:1
14:0
14:1
15:0
15:1
16:0
16:1
17:0
17:1
18:0
18:1
19:0
19:1
20:0
20:1
Carbon number

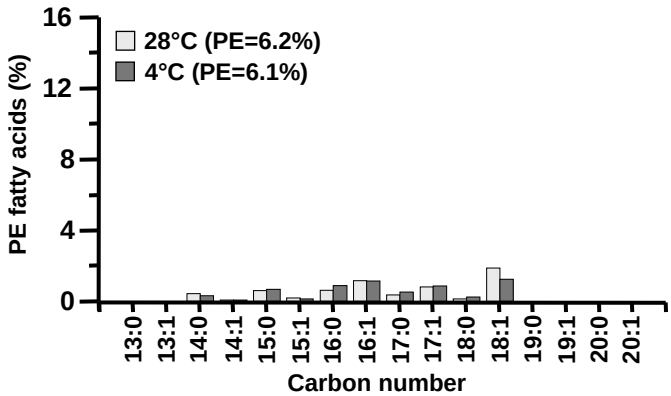

Sat. FA
Unsat. FA
PMMEFA[4°C]-PMMEFA[28°C] (%)
-2
-1
0
1
2
13:0
13:1
14:0
14:1
15:0
15:1
16:0
16:1
17:0
17:1
18:0
18:1
19:0
19:1
20:0
20:1
Carbon number

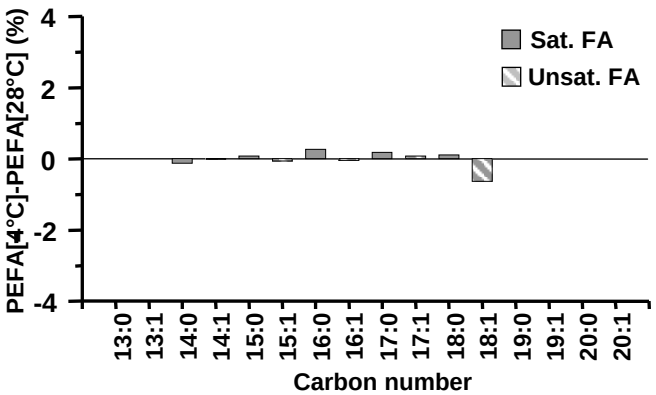

28°C (PC=62.7%)
4°C (PC=56.2%)
PC fatty acids (%)
0
4
8
12
16
20
13:0
13:1
14:0
14:1
15:0
15:1
16:0
16:1
17:0
17:1
18:0
18:1
19:0
19:1
20:0
20:1
Carbon number

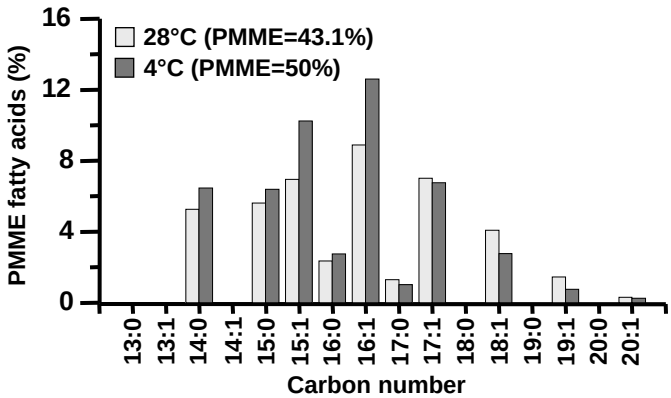

Sat. FA
Unsat. FA
PCFA[4°C]-PCFA[28°C] (%)
-2
-1
0
1
2
13:0
13:1
14:0
14:1
15:0
15:1
16:0
16:1
17:0
17:1
18:0
18:1
19:0
19:1
20:0
20:1
Carbon number

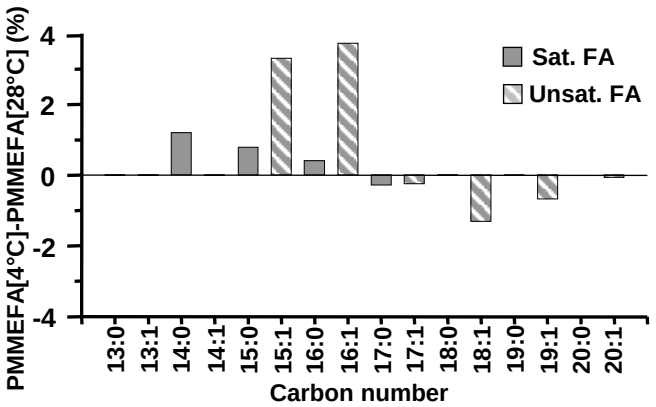

28°C (PG=9.9%)
4°C (PG=9.7%)
PG fatty acids (%)
Carbon number

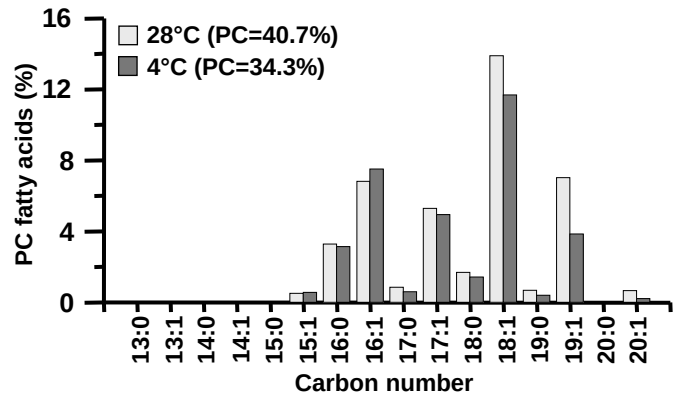

Sat. FA
Unsat. FA
PGFA[4°C]-PGFA[28°C] (%)
Carbon number

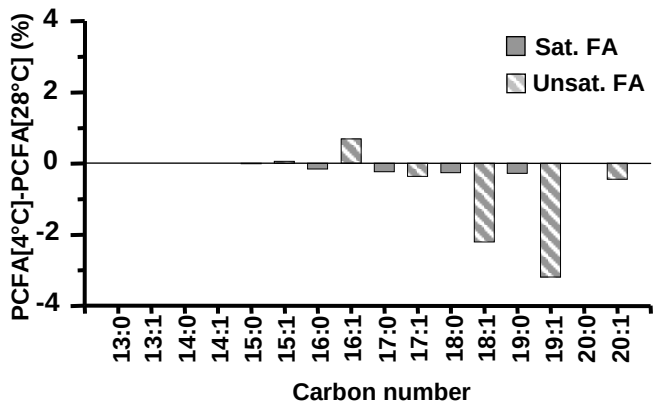

28°C (PE=6.2%)
4°C (PE=6.1%)
PE fatty acids (%)
Carbon number


Sat. FA
Unsat. FA
PEFA[4°C]-PEFA[28°C] (%)
Carbon number

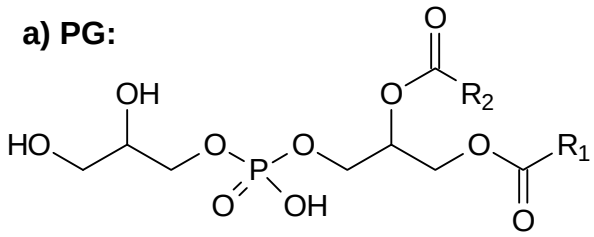

28°C (PMME=43.1%)
4°C (PMME=50%)
PMME fatty acids (%)
Carbon number


Sat. FA
Unsat. FA
PMMEFA[4°C]-PMMEFA[28°C] (%)
Carbon number

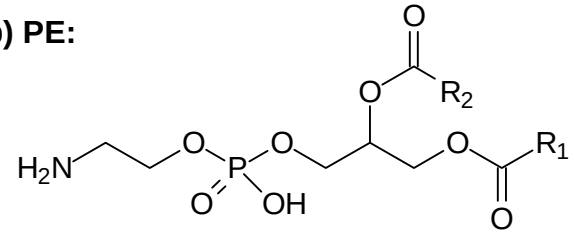

28°C (PC=40.7%)
4°C (PC=34.3%)
PC fatty acids (%)
Carbon number


Sat. FA
Unsat. FA
PCFA[4°C]-PCFA[28°C] (%)
Carbon number

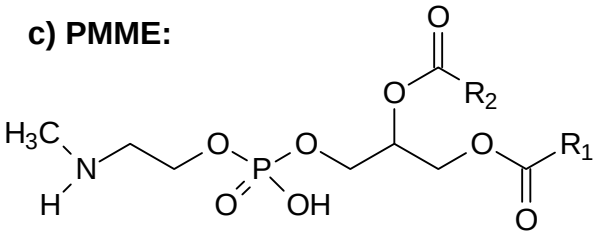
a) PG:



b) PE:



c) PMME:



d) PC: