

## Characterization of soil microflora on a successional glacier foreland in the high Arctic on Ellesmere Island, Nunavut, Canada using phospholipid fatty acid analysis

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**Abstract:** We investigated soil microbial biomass and community structure along a primary successional gradient after deglaciation in the high Arctic, at Ellesmere Island, Nunavut, Canada (80° 50' N, 82° 45' W). Soil samples were collected from five glacial moraines (M1 to M5) with different developmental periods. Time since the recession of glaciers at M1, M3, and M5 was estimated to be 300, 9000, and over 17000 years, respectively. Soil samples from five points in each moraine were subjected to phospholipid fatty acid (PLFA) analysis. Total PLFA content (an index of microbial biomass) in M1 was significantly lower than those in older moraines (M2–M5), whereas the content remained at an almost constant level from M2 to M5. Significant differences in PLFA composition (an index of microbial community structure) were also observed between M1 and older moraines (M2–M5); the proportion of straight chain saturated fatty acids in M1 was higher than those in older moraines (M2–M5), whereas those of branched fatty acids and unsaturated fatty acids in M1 were lower than those in older moraines (M2–M5). These results suggest that changes of microflora occurred in the early phase of succession after deglaciation and became stable thereafter. Microbial biomass had a positive correlation with soil carbon and nitrogen contents over the successional chronosequence, suggesting that development of soil microflora was affected in part by organic matter accumulation.

**key words:** high Arctic, microbial biomass, microbial community structure, phospholipid fatty acid (PLFA), primary succession after deglaciation

### Introduction

Glacial retreat provides new habitat for plant colonization and hence soil development. In the high Arctic where plant colonization takes place very slowly, various plant communities ranging from pioneer to later successional species are observed along a primary successional gradient within a glacier foreland. Successional changes of vegetation after glacial retreat in high Arctic regions and their relation to environmental conditions have been studied by a number of ecologists (e.g. Svoboda and Henry, 1987;

Minami and Kanda, 1995; Hodkinson *et al.*, 2003; Jones and Henry, 2003; Okitsu *et al.*, 2004).

In contrast, there are few studies that have focused on changes in soil microflora along a primary succession in a high Arctic glacier foreland. Soil microorganisms are thought to play an important role in soil formation and therefore in the progress of primary succession through decomposition and mineralization of organic matter. Bekku *et al.* (1999, 2004), studied soil respiration and substrate-induced respiration in a high Arctic glacial foreland in Ny-Ålesund, Svalbard and reported that soil microbial biomass and activities changed through successional chronosequences of thousands of years. In addition to microbial biomass, microbial community structure may also be important in controlling soil processes (Nakas and Klein, 1980; Cavigelli and Robertson, 2000; Balsler *et al.*, 2002). However, little is known about long-term changes in soil microflora (both biomass and composition) with the progress of primary succession after deglaciation in high Arctic regions.

One way to examine both the entire microbial biomass and community structure is phospholipid fatty acid (PLFA) analysis (Tunlid and White, 1992; Bardgett and McAlister, 1999; Bailey *et al.*, 2002). Phospholipids are a major component of all living cells and they decompose rapidly after cell death (White *et al.*, 1979). Composition of PLFAs is good indicator of the community structure of living soil microorganisms since different subsets of a community have different PLFA patterns (Tunlid and White, 1992). Although some ecologists have used this method to examine the difference in soil microflora on a successional glacier foreland (Ohtonen *et al.*, 1999; Bardgett and Walker, 2004), there is no study of soil PLFA in high Arctic regions.

This study aimed to clarify changes in microbial biomass and community structure using PLFA analysis on a relatively long successional chronosequence (more than 17000 years) in Ellesmere Island, a high Arctic area.

## Materials and methods

### Study site

The study area was the glacier foreland in the southern front of the Arklio Glacier, located near the mouth of the Oobloyah Valley, *ca.* 6 km east of Oobloyah Bay, Ellesmere Island, Nunavut, Canada (80°50'N, 82°45'W, Fig. 1). The climate in this area is represented by the weather station at Eureka (80°00'N, 85°56'W), located 130 km south of the study area. Annual mean temperature is -19.7°C and monthly mean temperatures of the warmest (July) and coldest (February) months are 3.3°C and -38.0°C, respectively. Annual precipitation is 64 mm (Atmospheric Environmental Service, 1982; after Kojima, 1994). The Arklio Glacier has developed at least five glacial moraines (moraines 1 to 5) with different developmental periods since the Last Glacial (Hasegawa *et al.*, 2004; Fig. 2). Moraine 1 (M1) is located near the edge of the Arklio Glacier, whereas moraine 5 (M5) is located in the outermost part of the study area. Moraines 2 to 4 (M2-M4) lie between M1 and M5 and retain clear moraine morphology. Times since the recession of glacier at M1, M3, and M5, estimated based on the geomorphological observations, weathering ring thickness, and lichenometry, were 300, 9000, and over 17000 years, respectively (Hasegawa *et al.*, 2004; Okitsu *et al.*, 2004).

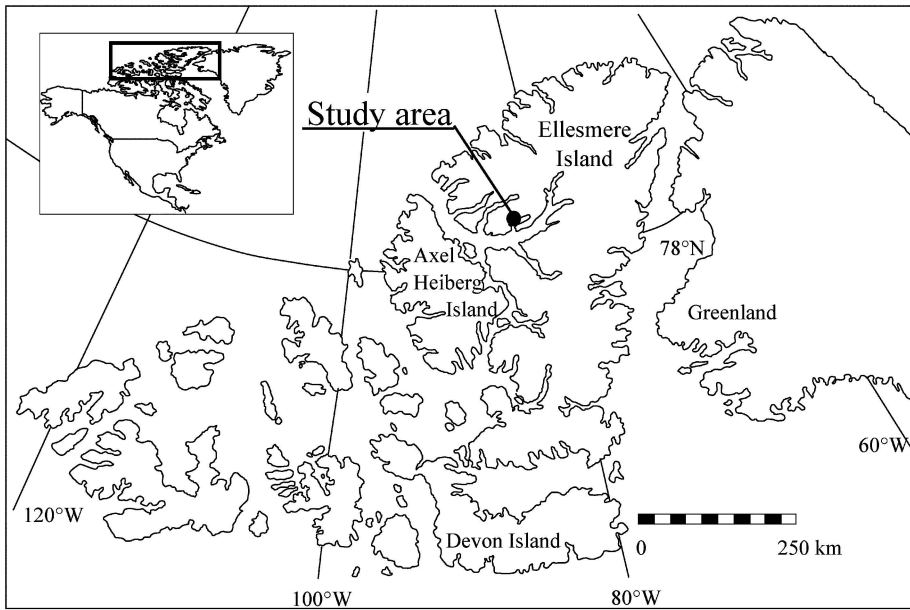


Fig. 1. Geographical location of the study area.

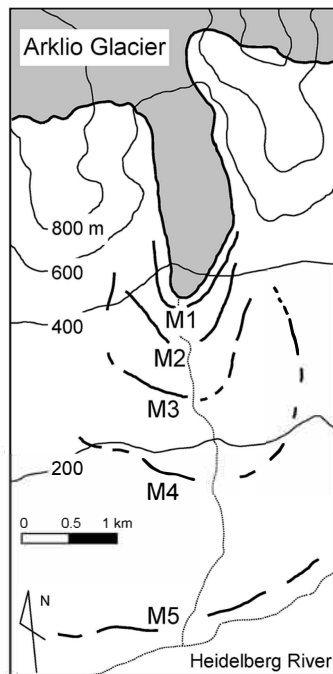


Fig. 2. Schematic representation of the distribution of the five moraines (M1–M5) (modified after Okitsu *et al.* (2004) and Hasegawa *et al.* (2004)).

More detailed topographic descriptions about this area appear in Hasegawa *et al.* (2004) and Okitsu *et al.* (2004).

M1 was almost bare ground covered with sharp-edged rocks, and the colonization of plants was very limited with coverage of plants including vascular plants, moss, and algae being less than 1% of the ground surface. The plant cover was higher in the other moraines, ranging from 40% in M2 to 61% in M5. In these moraines, *Salix arctica*, *Dryas integrifolia*, and *Cassiope tetragona* were the dominant plant species. Black soil crusts (soil-surface communities consisting of algae and cyanobacteria) are also an important vegetation cover in this area. More information about the vegetation on these moraines can be found in Osono *et al.* (2006).

### Soil sampling

In late July 2004, we set a 2×2 m plots on each moraine (M1–M5). Each plot contained plant species characteristic to each moraine as reported by Okitsu *et al.* (2004) and Osono *et al.* (2006). In each plot, soil samples were collected from five points (5×5 cm) that were chosen referring to the relative coverage of vegetation types within the plot. In M1, soil samples were collected from bare ground because there was little vegetation cover. In the other moraines (M2–M5), soil samples were collected after

Table 1. Soil characteristics in the study area. Mean value (SE).  $n = 5$ .

Moraine	M1	M2	M3	M4	M5
Water content (%) <sup>a</sup>					
0-0.5 cm	N.D.	N.D.	N.D.	N.D.	N.D.
0.5-2.0 cm	17.6 (8.6)	113.1 (6.5)	28.2 (8.8)	22.3 (6.0)	72.8 (60.6)
2.0-5.0 cm	5.8 (0.5)	116.1 (13.2)	45.6 (21.5)	22.9 (2.6)	30.68 (11.1)
Total carbon (mg g <sup>-1</sup> soil)					
0-0.5 cm	7.2 (0.5)	153.3 (34.6)	75.6 (23.6)	41.6 (21.0)	36.1 (10.0)
0.5-2.0 cm	8.6 (0.6)	141.4 (33.5)	50.0 (15.0)	46.3 (24.8)	18.2 (6.2)
2.0-5.0 cm	9.2 (0.8)	115.2 (27.8)	30.1 (7.3)	41.7 (20.3)	12.4 (4.1)
Total nitrogen (mg g <sup>-1</sup> soil)					
0-0.5 cm	0.26 (0.05)	11.3 (2.4)	5.2 (1.5)	2.7 (1.1)	2.4 (0.6)
0.5-2.0 cm	0.23 (0.04)	10.8 (2.4)	3.6 (1.0)	2.9 (1.3)	1.4 (0.4)
2.0-5.0 cm	0.25 (0.03)	9.0 (2.1)	2.2 (0.5)	2.6 (1.1)	1.0 (0.3)
C/N ratio					
0-0.5 cm	35.0 (9.9)	13.3 (0.6)	13.8 (0.9)	13.8 (1.0)	14.3 (0.6)
0.5-2.0 cm	45.7 (12.9)	12.8 (0.5)	13.5 (0.4)	14.7 (0.9)	13.0 (0.5)
2.0-5.0 cm	42.0 (9.5)	12.8 (0.3)	13.4 (0.2)	15.1 (0.8)	11.9 (0.5)
Dominant vegetation cover	–	Black soil crusts <i>Dryas integrifolia</i>	Black soil crusts <i>Dryas integrifolia</i> Moss	Black soil crusts <i>Dryas integrifolia</i> Moss <i>Cassiope tetragona</i>	Black soil crusts <i>Dryas integrifolia</i> Moss <i>Cassiope tetragona</i> Lichen

<sup>a</sup>Determined on August 11, 2004 by vacuum-drying,  $n=2-5$ .

N.D., not determined.

removing aboveground biomass and the organic layer (Table 1). The 0–0.5, 0.5–2, and 2–5 cm mineral soil layers in each sampling point were collected and put into paper bags. These samples were vacuum-dried in the field ( $<10^{\circ}\text{C}$ , within one week), and brought back to Japan at room temperature ( $5\text{--}20^{\circ}\text{C}$ ). The water contents of the soil samples were determined according to their weights before and after vacuum-drying. Then, samples were sieved ( $<2\text{ mm}$ ) to remove plant roots and gravel, and stored at  $-80^{\circ}\text{C}$  in a refrigerator until PLFA analysis. Total soil C and N were measured with a CN analyzer (Perkin-Elmer 2400 II, Perkin Elmer inc., Wellesley, MA, USA).

#### PLFA analysis

Total lipids including phospholipids were extracted from soil samples using the Bligh and Dyer (1959) extraction method, as modified by White *et al.* (1979) and Frostegård and Bååth (1996). Briefly, 1–3 g (dry weight) of soil was extracted with a chloroform–methanol–citrate buffer mixture (1:2:0.8). Lipids were separated into neutral lipids, glycolipids, and phospholipids on a silicic acid column (Sep-Pak<sup>TM</sup> plus silica, Waters, Milford, MA, USA) (Arao *et al.*, 2001). Phospholipids were esterified with HCl–Methanol Reagent (Tokyo Kasei Kogyo, Tokyo, Japan) (Stoffel *et al.*, 1959), and the resulting fatty acid methyl esters were separated on a Shimadzu GC-MS QP5000 (Shimadzu, Kyoto, Japan) equipped with a 30 m DB-5MS (Phenyl–Methyl/Silicone, J&W Scientific, Folsom, CA, USA) capillary column. Helium was used as the carrier gas. Peak areas were quantified by adding methyl nonadecanoate fatty acid (19:0) as an internal standard. The fatty acid nomenclature used was as described by Frostegård *et al.* (1993a). The total content of PLFAs (TotPLFAs) was used to indicate the total microbial biomass (Frostegård *et al.*, 1993b). As phospholipids are good indicators of the composition of the living soil microorganisms (White *et al.*, 1979), we use PLFA composition as an index of microbial community structure. PLFAs were classified into four categories (straight chain saturated fatty acids, branched chain fatty acids including cyclopropane fatty acids, unsaturated fatty acids, and 18:2 $\omega$ 6) and their proportion (mol%) was summed.

#### Statistical analysis

Data for total PLFA contents (nmol) and classified PLFA proportion (mol%) were subjected to two-way ANOVA to test for significant differences among the moraine and sampling depth. Rank order was determined by the Tukey–Kramer test.

Composition data of the major 18 PLFAs, described as mole percent, was standardized. These 18 PLFAs on the average constituted about 90% of TotPLFAs. Cluster analysis was applied to these data to clarify differences of microbial community structure with PAST software (Hammer *et al.*, 2001).

### Results and discussion

Both soil depth and moraines had significant effects on total PLFA (TotPLFAs) content (Table 2). TotPLFAs content was highest in the 0–0.5 cm soil depth for all moraines and tended to decrease with soil depth (Fig. 3). The content in the newest moraine (M1) was significantly smaller than those in older moraines (M2–M5) irrespec-

Table 2. Results of two-way ANOVA showing the effects of moraines and soil depth on TotPLFAs content ( $\text{nmol g}^{-1}$  soil).

Source	df	ms	F
Moraine	4	251182	6.50 ***
Depth	2	246105	6.37 **
Moraine $\times$ depth	8	25998	0.67
Error	52	38643	-

\*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$

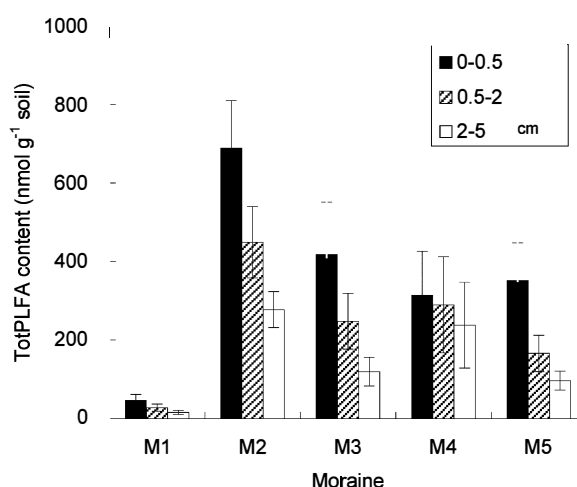


Fig. 3. TotPLFAs contents in soil as an index of microbial biomass of the five moraines (M1–M5).

tive of soil depth (Tukey–Kramer test,  $P < 0.05$ ). The content in M1 ( $15.8\text{--}46.4 \text{ nmol g}^{-1}$  soil) was similar to values reported for bare ground of a deglaciated area in Alaska ( $58^{\circ}\text{--}59^{\circ}\text{N}$ , Bardgett and Walker, 2004). In contrast, soil samples from M2 showed high TotPLFAs contents ( $278\text{--}689 \text{ nmol g}^{-1}$  soil) that were comparable to those in temperate grassland (e.g. Macdonald *et al.*, 2004). There were no significant differences in TotPLFAs content in all depths between older moraines (M2–M5) (Tukey–Kramer test,  $P > 0.05$ ).

These results suggest that a significant increase in microbial biomass occurred only in the early phase of the succession (between M1 and M2). This is in contrast to the study by Bekku *et al.* (1999) who reported that soil microbial biomass increased over the successional chronosequence in deglaciated areas in the high Arctic at Ny-Ålesund. This apparent difference may be partly due to the difference in the time scales of the successional chronosequences between the two study sites; the time since deglaciation exceeds 17000 years in our study, while that in the latter study was estimated to be thousands of years even at the oldest site (*cf.* Kume *et al.*, 1999).

In our study site, there was a high positive correlation between total soil C or N

content and TotPLFAs content throughout the successional chronosequence ( $P < 0.05$ ) (Fig. 4). Bekku *et al.* (1999) also reported a positive correlation between soil C or N content and microbial biomass in deglaciated areas in the high Arctic at Ny-Ålesund.

Table 3 and Fig. 5 show the composition of the 18 major PLFAs and the proportion of classified PLFAs, respectively. Soil depth had no significant effect on the pro-

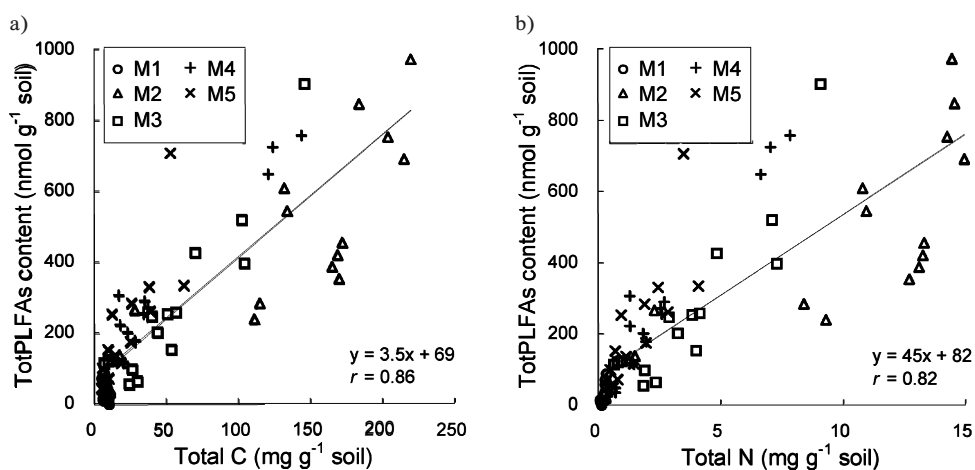


Fig. 4. Relationship between TotPLFAs contents and a) total carbon content or b) total nitrogen content in the soil.

Table 3. Composition of major 18 PLFAs (mol%). Mean value (SE).  $n = 5$ .

Moraine Depth (cm)	M1			M2			M3			M4			M5		
	0-0.5	0.5-2	2-5	0-0.5	0.5-2	2-5	0-0.5	0.5-2	2-5	0-0.5	0.5-2	2-5	0-0.5	0.5-2	2-5
<b>Straight chain</b>															
<b>saturated fatty acids</b>															
14:0	8.2	3.4	2.4	1.6	2.0	2.4	1.3	1.6	2.6	1.1	1.2	1.2	1.2	3.0	3.1
15:0	3.8	3.9	1.5	0.8	0.4	0.4	0.7	0.7	1.0	0.5	0.8	0.4	1.0	0.7	1.0
16:0	24.0	25.5	41.6	13.5	14.2	15.8	15.3	16.3	17.1	12.8	12.8	13.1	15.1	17.1	13.7
17:0	4.0	17.7	12.4	1.6	3.2	2.8	1.5	1.8	3.0	4.9	4.1	3.2	1.4	1.8	7.0
18:0	13.7	20.8	27.1	3.7	4.9	6.4	5.5	6.5	8.0	4.5	5.2	6.2	4.1	6.4	8.6
20:0	-	-	-	1.1	-	2.9	-	0.6	-	0.6	0.7	1.6	0.3	0.8	-
<b>Branched chain fatty acids</b>															
<b>&amp; Cyclopropane fatty acid</b>															
a13:0	-	0.5	-	1.1	1.2	1.0	0.9	1.3	2.0	0.4	0.2	0.5	0.2	0.0	1.3
i14:0	-	0.7	-	1.6	1.8	2.3	1.2	1.4	0.4	0.2	0.5	0.6	1.5	0.3	0.4
i15:0	1.1	0.4	0.2	10.0	9.8	8.9	8.6	8.5	8.9	10.5	9.0	8.4	10.1	10.0	7.7
a15:0	1.5	1.0	0.7	6.4	6.8	7.0	5.7	5.9	6.4	5.7	5.5	5.2	6.0	6.0	5.1
i16:0	1.6	1.9	2.9	3.8	3.9	4.1	3.3	3.6	4.1	3.1	2.4	2.0	3.3	3.9	2.4
i17:0	0.3	0.3	-	2.6	3.2	3.1	1.7	1.8	1.8	2.1	2.2	2.2	2.4	2.8	3.2
a17:0	-	-	-	2.9	3.1	5.0	2.0	2.4	0.9	3.2	1.6	3.0	1.9	2.1	4.8
cy17:0	-	-	-	-	-	-	-	0.8	-	0.4	0.5	0.4	-	-	-
<b>Unsaturated fatty acids</b>															
16:1 $\omega$ 7	6.1	1.9	0.9	9.6	9.2	8.0	8.9	7.5	5.6	7.3	6.4	7.1	11.0	12.3	7.6
18:1 $\omega$ 9	8.9	1.5	0.8	7.5	5.4	3.9	6.6	5.7	4.1	6.5	7.1	7.0	6.8	4.3	3.6
18:1 $\omega$ 7	2.8	3.6	0.7	13.0	12.0	9.2	12.6	11.6	9.9	16.6	14.8	14.2	11.9	8.5	7.9
<b>Fungal fatty acids</b>															
18:2 $\omega$ 6	5.7	3.8	0.0	7.9	7.6	6.9	12.8	13.7	11.6	10.0	12.6	13.8	11.2	11.2	10.6

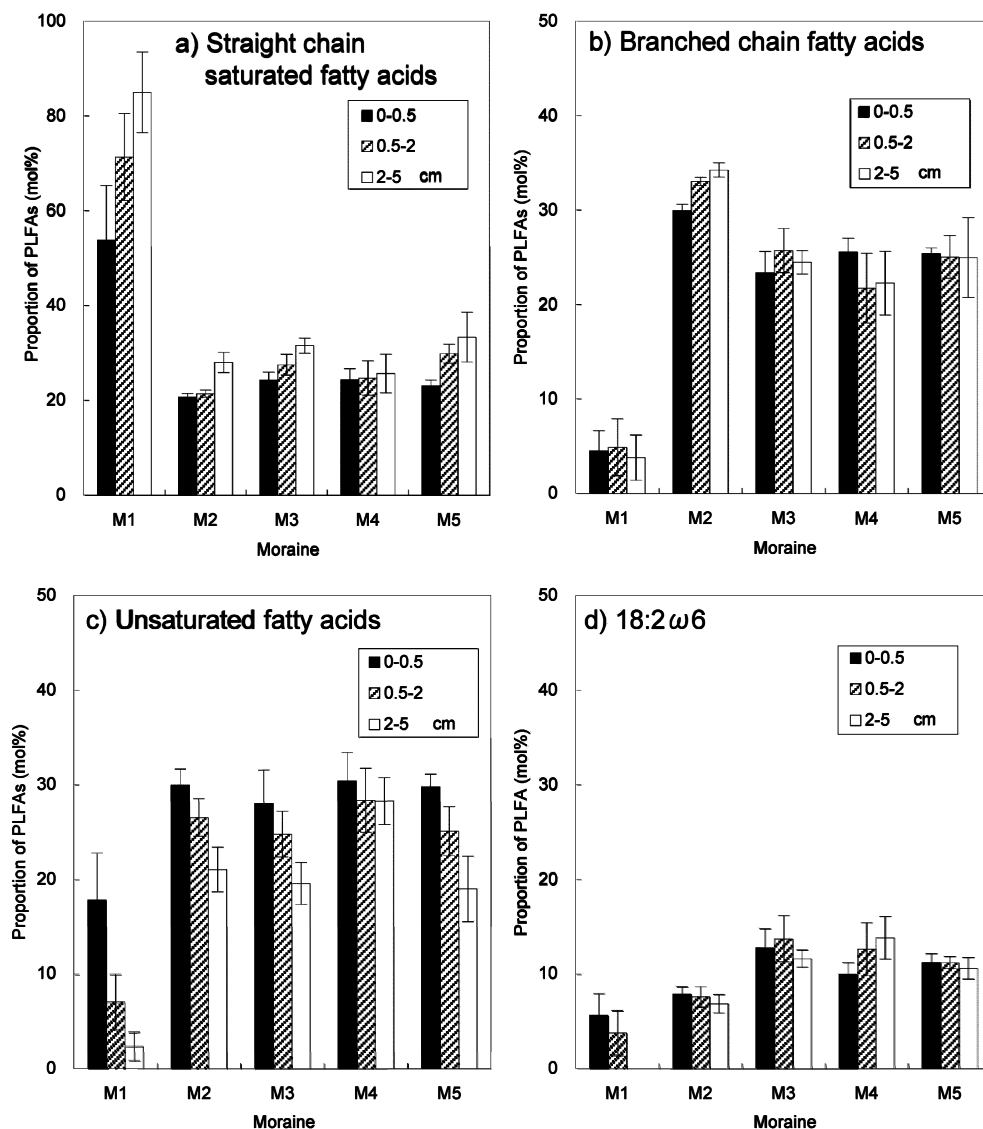


Fig. 5. Proportion of categorized PLFAs in soil of the five moraines (M1–M5); a) straight chain saturated fatty acids, b) branched chain fatty acids (including cyclopropane fatty acids), c) unsaturated fatty acids, and d) 18:2 $\omega$ 6.

portion of branched chain fatty acids and that of 18:2 $\omega$ 6 (Table 4 and Fig. 5). In contrast, soil depth had a significant effect on the proportion of straight chain saturated fatty acids and that of unsaturated fatty acids (Table 4); that of straight chain saturated fatty acids in M2 and M3 increased significantly and that of unsaturated fatty acids in M1 and M5 decreased with soil depth (Tukey–Kramer test,  $P < 0.05$ ). However, in most cases, soil depth has little effect on PLFA composition. In contrast, there are significant



differences in the proportion of classified fatty acids among moraines (Table 4 and Fig. 5). The proportion of straight chain saturated fatty acids in M1 was significantly higher than those in older moraines (M2–M5) irrespective of soil depth (Tukey–Kramer test,  $P < 0.05$ ). On the other hand, the proportions of branched chain fatty acids and unsaturated fatty acids in M1 were significantly lower than those in older moraines (M2–M5) in all soil depths except for that of unsaturated fatty acids in 0–0.5 cm. The proportion of 18:2 $\omega$ 6, an index of fungal biomass (Federle, 1986), in M1 was also significantly lower than that in M3.

Figure 6 shows the results of hierarchical cluster analyses, made on the averaged data sets of the PLFA composition of each sampling point (average of 0–5 cm depth).

Table 4. Results of two-way ANOVA showing the effects of moraines and soil depth on the proportion of categorized PLFAs (mol%).

Source	Straight chain saturated fatty acids			Branched chain fatty acids			Unsaturated fatty acids			18:2 $\omega$ 6		
	df	ms	F	df	ms	F	df	ms	F	df	ms	F
Moraine	4	1.17	30.85 ***	4	0.17	58.89 ***	4	0.09	22.56 ***	4	0.02	17.40 ***
Depth	2	0.17	4.49 *	2	<0.01	0.03	2	0.06	13.31 ***	2	<0.01	0.72
Moraine $\times$ depth	8	0.06	1.59	8	<0.01	0.50	8	<0.01	0.84	8	<0.01	1.07
Error	60	0.04	-	60	<0.01	-	60	<0.01	-	60	<0.01	-

\*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$

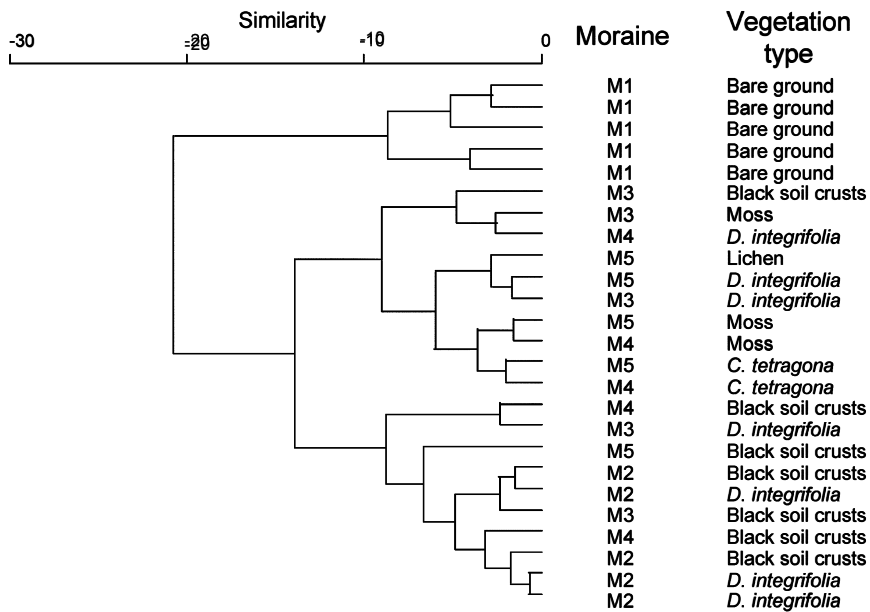


Fig. 6. Results of hierarchical cluster analysis using Ward's method (Ward, 1963) of PLFA composition data (mol%).

There were two primary clusters; one contained only soils from bare ground in M1, and another cluster contained two secondary clusters. Of the two secondary clusters, one contained soil from the other four moraines (M2, M3, M4, and M5), while the other contain soil from the older moraines (M3, M4, and M5).

Significant differences in the proportions of classified PLFAs indicate that the microbial community structure in M1 differed widely from those in the older moraines (M2–M5) (Fig. 5). In contrast, the older moraines (M2–M5) showed similar proportions of these classified PLFAs irrespective of soil depth, suggesting that they had similar microbial community structures. Hierarchical cluster analysis also showed that the community structure in M1 was clearly differentiated from those in older moraines (M2–M5) (Fig. 6).

These results suggest that the shift in microbial community structure as well as the increase in microbial biomass occurred mainly in the early phase of the succession (between M1 and M2). Ohtonen *et al.* (1999) reported that microbial community structure in soil under vegetation was clearly different from that in nonvegetated soil in a recently deglaciated area (48°N, 20–80 years). Thus, the characteristic microbial community structure observed in M1 in our study is maybe explained by the absence of vegetation cover.

The pattern of development in microflora in this area is somewhat different from that of vegetation. Okitsu *et al.* (2004) reported that vegetation development occurred over the successional chronosequence in this area (M1–M5). In contrast, microbial biomass and community structure became stable in the late phase of succession (M3–M5). Although the effect of vegetation type on microflora was not clear, microbial biomass had a positive correlation with soil carbon or nitrogen content over the successional chronosequence. This suggests that development of soil microflora in a glacier foreland in the high arctic was affected more by organic matter accumulation rather than by plant species composition. In this study area, a significant increase in soil organic matter was observed only in the early phase of succession (M1–M2) (*cf.* Table 1). This may partly explain why the pattern of development in soil microflora was different from that in vegetation.

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