

# Microbial biomass and community composition in soils from Surtsey, Iceland, studied using phospholipid fatty acid analysis

MORTEN KLAMER<sup>1</sup>, ANDREA SPONRING<sup>2</sup> & ERLAND BÅÅTH<sup>3</sup>

<sup>1</sup>Botanical Institute, University of Copenhagen, Øster Farimagsgade 2 D, DK-1353 Copenhagen K, Denmark.

<sup>2</sup>Institut für Mikrobiologie, Universität Innsbruck, Technikerstrasse 25, A-6020 Innsbruck, Austria.

<sup>3</sup>Dept. of Microbial Ecology, Lund University, S-223 26 Lund, Sweden.

## ABSTRACT

Soil samples from Surtsey were analysed for chemical properties, number of fungal colony forming units and phospholipid fatty acid (PLFA) profiles. The temperature in the samples varied from 16 to 74°C. The microbial biomass in the samples was rather low, which may be due to environmental stress. There was a separation of the microbial communities in hot and temperate habitats as evaluated by principal component analysis. The dominating phospholipid fatty acids in hot soils were branched fatty acids, which primarily are found in Gram-positive bacteria. There are also a significant decrease in fatty acids characteristic of Gram-negative bacteria with increasing temperature.

## INTRODUCTION

One method to estimate the total microbial biomass is the extraction and quantification of phospholipid fatty acids (PLFAs), which are located in cell membranes of all living organisms (Ratledge & Wilkinson 1988). Results from the PLFA technique have been shown to correlate well with other methods for estimating microbial biomass (Federle 1986, Frostegård *et al.* 1991, Tunlid & White 1992, Zelles *et al.* 1995), and the turnover rates of PLFA after death of the organisms seem to be rather fast (White *et al.* 1979, Tollefson & McKercher 1983, Klamer & Bååth 1998).

The major advantage of using this technique is, however, the possibility to separate the microbial biomass into major taxonomic groups. This can be achieved because different groups of organisms have PLFAs, which are almost exclusively found within the group. For example 10Me16:0 (for the nomenclature of the PLFA see Materials and Methods) has been suggested as marker for *Desulfobacter* spp. in marine sediments (Dowling *et al.* 1986), and 10Me18:0 has been suggested as a

marker for actinomycetes (Tunlid & White 1992). 18:26,9 is almost exclusively found in eucaryotes, mainly in fungi and plants (Federle 1986, Wellburn *et al.* 1994, Zelles 1997), although it has been found in some marine bacteria (Johns & Perry 1977). In the same way one group of PLFAs are mainly found in Gram-negative (Federle 1986, Zelles 1997) and one in Gram-positive bacteria (O'Leary & Wilkinson 1988, Tunlid & White 1992, Zelles 1997). Thus compared to classic methods like, e.g. direct counts of cells and hyphae (Domsch *et al.* 1979, Elmholt & Kjeller 1987) and fumigation-extraction techniques (Jenkinson & Powlson 1976, Anderson & Domsch 1978), it is possible to estimate the viable biomass of both fungi and bacteria with the same technique and even within the same sample (Lechevalier & Lechevalier 1988, Frostegård & Bååth 1996).

Therefore the PLFA technique has been widely used to estimate changes in the composition of the microbial community in natural systems, e.g. aquatic systems (King *et al.* 1977, Gillan & Hogg 1984, Kieft *et al.* 1997), soils (Zelles *et al.* 1994, Bååth *et al.*

1995, Frostegård & Bååth 1996, Zogg *et al.* 1997), manure "hot spots" (Frostegård *et al.* 1997) and composts (Hellmann *et al.* 1997, Herrmann & Shann 1997, Klammer & Bååth 1998). But to our knowledge this technique has not previously been used to investigate soils with high temperatures.

In the studies of composts mentioned above the microbial communities were dominated by PLFAs common in Gram-positive bacteria during the heating phase, while marker PLFAs for fungi and Gram-negative bacteria were low or absent (see, e.g. Klammer & Bååth 1998). The marker PLFA for actinomyces was at a constant low level during the composting experiments. The changes in PLFA profile with changes in temperature can be caused by two mechanisms. Either it is change in the composition of the cell membranes of the microorganisms present or probably more important it is a shift in the composition of the microbial community. There is some evidence that microorganisms can change the composition of the PLFAs in the cell membranes in order to tolerate increased temperature (Sumner & Morgan 1969, Mumma *et al.* 1971, Oshima & Miyagawa 1974, Suutari *et al.* 1990). These changes can be either chain elongation (Oshima & Miyagawa 1974), more saturation of the fatty acids (Mumma *et al.* 1971) or changes in the ratio of *iso/anteiso* branched fatty acids. These characteristics can also be found in thermophilic organisms. Thus, by just looking at these ratios it can not be elucidated if changes are due to other organisms thriving in high temperature environments, or if they are due to cell membrane changes in the same organisms that are present also in habitats with lower temperatures. However, Kaneda (1991) divided the membrane lipids in two families on basis of their synthetic pathways. One group consisted of straight-chain fatty acids, and a second group having branched fatty acids. Thus a change in the composition of PLFA to a profile containing, e.g. more branched fatty acids must be caused by a change in the composition of microorganisms in the community.

The aim of this study was to measure the microbial biomass in natural high temperature soils, and to investigate whether the changes in composition of the microbial community due to changes in temperature could be detected by the PLFA technique. In order to obtain this the PLFA profile of soil samples ranging from 16 to 74°C in temperature were compared. The PLFA profiles were evaluated by studying the main chain length of the PLFA by calculating the ratio C16/C18, the degree of unsaturation of the PLFA and the ratio between *iso* and *anteiso* branched PLFA. Finally the profiles from all

samples were subject to a principal component analysis, and the changes in different subsets of the microbial community were evaluated by comparing marker PLFAs for the groups.

## MATERIALS AND METHODS

### *Sampling*

During the summer 1996 soil samples were collected at Surtsey in an area between Surtur I and II close to a crevice. Samples were taken from areas dominated with *Honkenya peploides* (L.) Ehrh., bare soil and moss, respectively. In 1997 additional samples were collected from Surtsey and Japan. On Surtsey the sample location was also between Surtur I and II, but south to the sample area of 1996. This area was characterised by scattered vegetation of *H. peploides* and *Puccinellia distans* (L.) Parl., total cover was less than 5%. The distance between hot and temperate samples was about 5 m. The soil samples from Japan were collected at the Zigokudani hot springs, Yamanouchi, Nagano, an area dominated by various coniferous trees.

The samples were air dried and stored at 5°C until analysed.

### *Chemical and fungal analysis*

pH and conductivity were measured by mixing 5 g soil with 25 ml deionised water, and at least 24 hours of extraction. The total amount of nitrogen (totN) was measured on a Leco automatic nitrogen analyser (FP-428). The total amount of carbon (totC) was calculated from loss on ignition after heating at 550°C for 6 hours, assuming the organic matter contained 50% carbon.

From the samples collected in 1996 the number of fungal colony forming units (CFU) was counted by using the soil plate technique (Warcup 1950). 0.1 g soil was plated in petri dishes and 15 ml malt extract agar (20 g/l malt extract, 15 g/l agar and 150 ppm penicillin/streptomycin) was added. The Petri dishes were incubated at 24 and 40°C in the dark.

### *Phospholipid fatty acid analysis*

The PLFA extraction, fractionation, mild alkaline methanolysis and GC analysis used here were described in detail by Frostegård *et al.* (1991, 1993). Shortly, lipids were extracted from the soil samples in a one-phase mixture of chloroform-methanol-citrate buffer and the polar lipids were separated using silicic acid columns, followed by a mild alkaline methanolysis to form fatty acid methyl esters before GC analysis.

Fatty acids were designated in terms of total number of carbon atoms:number of double bonds, followed by the position of the double bond from the methyl end of the molecule. The prefixes a and i indicate *anteiso*- and *iso*-branching, cy indicates a cyclopropane fatty acid and methyl branching (Me) is indicated as the position of the methyl group from the carboxyl end of the chain.

The sum of the following fatty acids was considered to represent Gram-positive bacteria: i15:0, a15:0, i16:0, i17:0, a17:0 and 10Me17:0. The Gram-negative bacteria were represented by the sum of 16:17t, 16:15, cy17:0, 18:17 and cy 19:0. As marker for actinomycetes and fungi, 10Me18:0 and 18:26.9, respectively, were used.

The C16/C18 ratio was obtained by summarizing all PLFAs with a chain length of 16 and 18 carbon molecules, respectively, and calculate the ratio. The degree of unsaturation was calculated using:  $(12(\text{mol\% monoene}) + 22(\text{mol\% diene}) + 32(\text{mol\% triene}))/100$ .

The principal component analysis was obtained by using the mole percent data and scaling each variable to unit variance.

## RESULTS

The soil samples used in this study together with temperature, chemical and microbial properties are listed in Table 1 and 2. The amount of organic matter and nitrogen in the samples from Surtsey is low compared to the samples from Japan. pH and conductivity in the samples from Surtsey showed little variation, while sample no. 16 and 17 from Japan had low pH values and high conductivity, which probably was due to high amounts of sulphur in these samples.

Table 1. Location, temperature and number of colony forming units of soil samples from Surtsey, 1996.

Sample no.	Location	Temp. (°C)	CFU, 24°C (g soil-1)	CFU, 40°C (g soil-1)
32	Surtsey, bare soil	21	45	20
34	Surtsey, bare soil	21	23	13
33	Surtsey, bare soil	26	130	125
31	Surtsey, bare soil	43	26	20
35	Surtsey, moss	49	230	240
37	Surtsey, <i>Honkenya</i>	16	20	10
39	Surtsey, <i>Honkenya</i>	16	6	10
41	Surtsey, <i>Honkenya</i>	17	n.d.	56
38	Surtsey, <i>Honkenya</i>	20	93	50
40	Surtsey, <i>Honkenya</i>	25	60	63
36	Surtsey, <i>Honkenya</i>	26	60	43

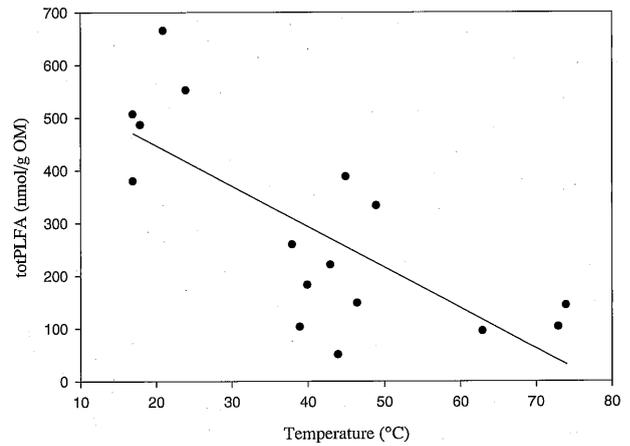


Figure 1. Total amounts of phospholipid fatty acids found in soil samples from 1997 with different temperatures.

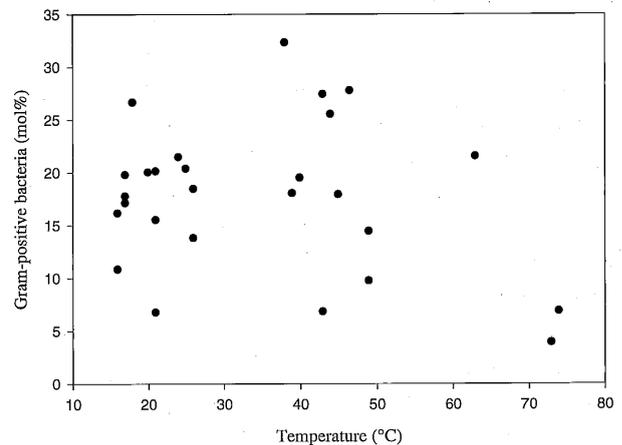


Figure 2. The relative amount of PLFAs characteristic of Gram-positive bacteria found in soil samples with different temperatures.

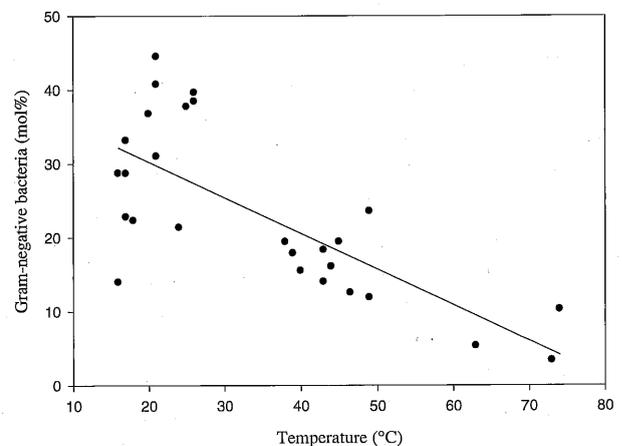


Figure 3. The relative amount of PLFAs characteristic of Gram-negative bacteria found in soil samples with different temperatures.

Table 2. Location, temperature and chemical analysis of soil samples collected in 1997.

Sample no.	Location	Temp. (°C)	totC (% of dw)	totN (% of dw)	C/N ratio	pH	Conductivity (µS)
1	Surtsey 1	63	1.4	0.06	24.2	7.8	129
2	Surtsey 2	73	1.2	0.12	9.7	7.6	91
3	Surtsey 3	61	0.8	0.10	7.3	7.5	70
4	Surtsey 4	49	0.6	0.06	9.6	7.1	41
5	Surtsey 5	45	0.5	0.12	4.3	7.2	56
6	Surtsey 6	74	1.1	0.10	11.6	7.6	90
7	Surtsey 7	21	0.2	0.06	3.9	7.3	49
8	Surtsey 8	17	0.3	0.05	5.9	7.2	70
9	Surtsey 9	17	0.3	0.05	7.3	7.2	57
10	Surtsey 10	18	0.3	0.04	7.8	7.4	58
11	Surtsey 11	24	0.3	0.01	32.1	7.4	56
12	Surtsey 12	17	0.3	0.01	20.7	7.3	59
16	Japan 1	44	7.5	0.32	23.4	2.4	9320
17	Japan 1	38	7.4	0.63	12.0	3.4	4940
18	Japan 2	40	3.9	0.22	17.9	4.9	451
19	Japan 3	43	4.6	0.22	20.9	7.2	146
20	Japan 3	39	2.4	0.09	27.3	4.1	252
21	Japan 4	47	4.7	0.13	36.9	6.9	32

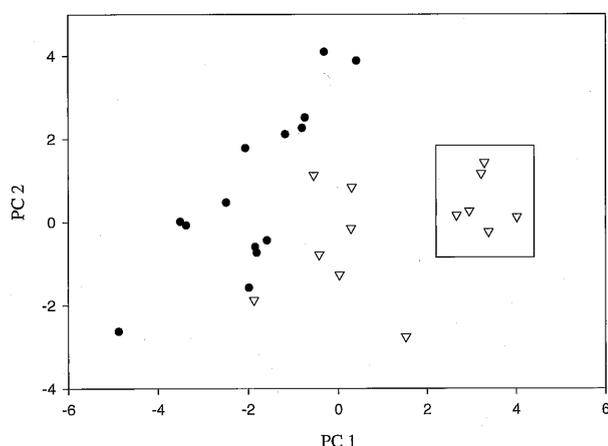


Figure 4. Principal component analysis of the soil samples. (•) samples in the temperature range 16-26°C. (▽) samples in the temperature range 38-74°C. The samples in the frame are from Japan.

In total 40 different PLFAs were detected and 34 were identified on basis of their relative retention time to the standard (19:0). In Fig. 1 the total amount of PLFA per g organic matter (nmol/g OM) found in each sample is plotted against temperature for the samples collected in 1997. There was a significant decrease in microbial biomass with increasing temperature ( $r^2=0.59$ ,  $p<0.05$ ). Sample no. 3 and 12 were omitted, since the amount of the most usual fatty acid, 16:0, was very low.

The marker PLFAs for Gram-positive bacteria had a tendency to increase in relative amount in the temperature interval 40-50°C (Fig. 2), although there was a large variation. The same

patterns were observed for the marker PLFA for actinomycetes (data not shown).

A significant decrease in the relative amount of marker PLFAs for Gram-negative bacteria with increasing temperature was observed ( $r^2=0.52$ ,  $p<0.05$ ) (Fig. 3).

A principal component analysis based on the PLFA profiles of all the samples is shown in Fig. 4. A clear separation of the temperate and the high temperature soil samples was demonstrated with the temperate samples to the left and the high temperature samples to the right. The first principal component explained 20.0% of the variation and the second 12.8%. There was a significant correlation between principal component one and temperature ( $r^2=0.44$ ,  $p<0.05$ ), indicating that the separation along PC 1 can be explained at least partly by the changes in temperature. In addition the samples from Japan were rather close, indicating a similar microbial community in these samples. The degree of unsaturation showed a significant negative correlation with temperature ( $r^2=0.46$ ,  $p<0.05$ ) (Fig. 5), while C16/C18 and *iso/anteiso* ratios did not show any clear relationship with temperature (data not shown).

## DISCUSSION

The total amounts of PLFA found in these soils (Fig. 1) were lower than normal. For example, Frostegård & Bååth (1996) studied a range of soils and found between 374 and 4694 nmol PLFA/g OM (mean around 2000 nmol/g OM). Using a conversion factor of 340 nmol PLFA/mg

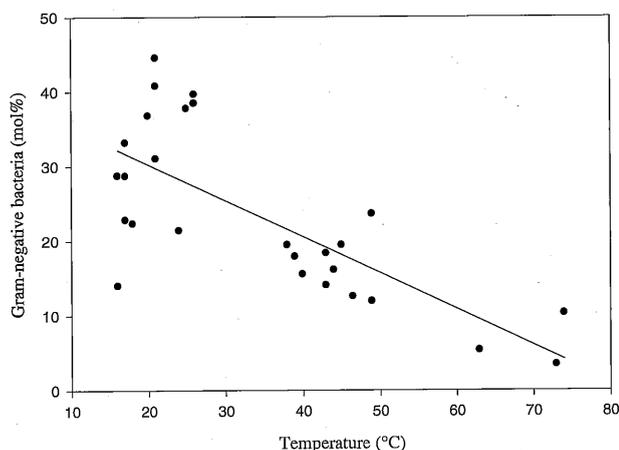


Figure 5. Correlation between the degree of unsaturation of the PLFA profiles found in soils and temperature. Sample no. 7 excluded, hence it has a degree of unsaturation of 1.33.

microbial biomass carbon and 50 % carbon of the biomass dry weight (Frostegård *et al.* 1991), the more temperate soils had around 3 mg microbial biomass decreasing to about 1 mg/g OM in the soils at higher temperature. This is very low compared to normal soil values of 10 to 30 mg/g OM (Wardle 1992). This might indicate that the soil organisms had a stressful situation even in the more temperate soils, and that increased temperatures made these conditions even more pronounced.

There was a clear separation of the temperate and high temperature samples, as seen from the PCA (Fig. 4). It is also interesting that the samples from Japan all clustered together, despite the large variation in chemical parameters (Table 2). This indicates that temperature was the main factor determining the composition of the microbial community, despite large variation also in for example pH and organic matter content between samples.

The results from this study indicate that Gram-positive bacteria are common not only in transient thermophilic habitats like composts, but also in more stable hot environments, while Gram-negative bacteria decrease in relative amount with increasing temperature. These findings are supported by data from traditional isolation techniques (see, e.g. Brock 1978, Strom 1985).

The PLFA marker for fungal presence, 18:2w6,9, constituted from 0 to 10 mol% in all the samples, without a clear relationship to temperature. This is probably caused by the fact that this PLFA also is present in plants and that a very limited number of fungi are present in the

soil of Surtsey. This was illustrated by the fact that in the samples from bare soil 18:2w6,9 was absent, and a low number of fungal CFU was found (Table 1).

The degree of unsaturation seemed to be a good indicator for the temperature adaption in the community, while the ratio's C16/C18 and *iso/anteiso* did not correlate. This is partly in agreement with the findings by Klamer & Bååth (1998), who studied the PLFA profile in composts, and found good correlation between degree of unsaturation and temperature, while *iso/anteiso* ratios did not correlate.

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