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Ecosystem properties and microbial community changes in primary succession on a glacier forefront

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Abstract We studied microbial community composition in a primary successional chronosequence on the forefront of Lyman Glacier, Washington, United States. We sampled microbial communities in soil from nonvegetated areas and under the canopies of mycorrhizal and nonmycorrhizal plants from 20- to 80-year-old zones along the successional gradient. Three independent measures of microbial biomass were used: substrate-induced respiration (SIR), phospholipid fatty acid (PLFA) analysis, and direct microscopic counts. All methods indicated that biomass increased over successional time in the nonvegetated soil. PLFA analysis indicated that the microbial biomass was greater under the plant canopies than in the nonvegetated soils; the microbial community composition was clearly different between these two types of soils. Over the successional gradient, the microbial community shifted from bacterial-dominated to fungal-dominated. Microbial respiration increased while specific activity (respiration per unit biomass) decreased in nonvegetated soils over the successional gradient. We proposed and evaluated new parameters for estimating the C use efficiency of the soil microbial community: "Max" indicates the maximal respiration rate and "Acc" the total C released from the sample after a standard amount of substrate is added. These, as well as the corresponding specific activities (calculated as Max and Acc per unit biomass), decreased sharply over the successional gradient. Our study suggests that during the early stages of succession the microbial community cannot incorporate all the added substrate into its biomass, but rapidly increases its respiration. The later-stage microbial community cannot reach as high a rate of respiration per unit biomass but remains in an "energy-saving state," accumulating C to its biomass.

Key words C use efficiency · Chronosequence · Metabolic quotient · Microbial biomass · Phospholipid fatty acid

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Introduction

Several authors (e.g. Clements 1916; Odum 1969; Peet 1992) have predicted that in the early stages of primary succession, at the time of initial plant recruitment and establishment, biomass, diversity and production will be low. N is often limiting during initial community development (Chapin et al. 1994) but at later stages soil organic matter builds up and this is accompanied by an increase in soil moisture and total N (Peet 1992; Chapin et al. 1994). Insam and Haselwandter (1989) proposed that during ecosystem succession soil microbial communities (which govern decomposition) become more efficient in their energy use, i.e. they incorporate a higher proportion of C into the biomass. At early stages C loss from the ecosystem due to respiration is significant and this loss declines over time. This is inferred from the decreasing respiration to biomass ratio (the metabolic quotient or qCO_2) that is sometimes observed during

initial stages of succession (Insam and Haselwandter 1989; Anderson 1994). Wardle and Ghani (1995) criticized this approach, as in several cases this variable has been found to respond unpredictably to ecosystem development because effects of stress are confounded with those of disturbance (e.g. drought and decrease in C input to soil, respectively, due to grazing), as shown by Ohtonen and Väre (1998). However, this variable still has value as a relative measure of how efficiently the soil microbial biomass is utilizing C resources, and of the degree of substrate limitation for the soil microbial biomass (Wardle and Ghani 1995). So far, no attempts have been made to use variables other than qCO_2 for describing C balance of the microbial community in the early stages of primary succession, but in man-made ecosystems microbial C relative to soil organic C (C_{mic}/C_{org}) has indicative value in describing ecosystem development (Insam and Domsch 1988; Ohtonen et al. 1992). As heterotrophic microorganisms totally depend on the C fixed by autotrophic organisms and are often substrate-specific, their succession could be expected to parallel the succession of the plant community. However, very few studies have linked plant population dynamics to changes in ecosystem properties by including the associated succession of soil organisms (Frankland 1992, 1998).

Insam and Haselwandter (1989) and Anderson (1994) present Odum's theory (Odum 1969) of ecosystem development and Connell and Slatyer's facilitation model (Connell and Slatyer 1977) as potential explanations for the development of the soil microbial community during primary succession. They associate the decrease in qCO_2 with increasing ecosystem development to the formation of more favourable conditions for microbial survival, and this reduces the maintenance energy required. This theory may be incomplete, however, and changes in microbial community structure and competition or other interactions between microbes and plants may contribute substantially to the patterns of resource utilization and processes in the microbial communities (Kaye and Hart 1997).

In the past decade new techniques have been introduced for studying the structure of soil microbial community, e.g. the ester-linked phospholipid fatty acid (PLFA) composition in a soil sample (Tunlid and White 1992; Frostegård and Bååth 1996). Subsets of the microbial community have different PLFA patterns, so it is possible to directly characterize the features of the microbial community in natural habitats. This approach makes an important contribution to understanding of the microbial community because less than 5% of the soil bacteria can be cultured (Bakken 1985), and because genetic diversity of the total microbial community may be 200 times higher than the diversity of isolated bacteria (Torsvik et al. 1994). However, simultaneous consideration of microbial communities and activities is necessary for better understanding of ecosystem-level processes such as energy transfer in the ecosystem.

In this study we characterized the communities of soil microorganisms as well as their activities along a primary successional vegetation gradient on a retreating glacier forefront. By analysing microbial communities associated with barren, i.e. non-vegetated, soil, and with soil under the canopies of mycorrhizal and non-mycorrhizal plants during ecosystem development, we attempted to relate the variable soil environment to the structure and function of the soil microbial community. The specific hypotheses we tested are that:

1. C use efficiency (i.e. allocation of C to biomass versus respiration) of the microbial community increases as succession proceeds, as proposed by Insam and Haselwandter (1989).
2. Changes in C use efficiency are related to the microbial community structure regardless of whether structure is assessed in terms of the bacterial/fungal ratios or in terms of the PLFA profile of the soil.
3. The structure of the microbial community in barren soil is different from that in rhizosphere soils.

Materials and methods

Study area

Lyman Glacier is located in the Glacier Peak Wilderness in the Wenatchee National Forest of Washington State in the North Cascades Range, at 48°10′14″N, 120°53′44″W, at an elevation of about 1800 m. The exposed forefront is 1100 m long and consists of heterogeneous glacial till ranging from clay-sized particles to boulders, intermingled with deposits of glacial-fluvial sediments (Jumpponen et al. 1998).

The glacial forefront was divided into the following vegetational phases based on the classification of Jumpponen et al. (1998):

1. A 20- to 30-year-old phase characterized by scattered individuals or small patches of the early seral plant species *Juncus drummondii*, *J. mertensianus*, *Luzula piperi*, *Saxifraga ferruginea* and *S. tolmiei*
2. A 30- to 50-year-old phase characterized by the same early seral species as in phase 1, and in addition scattered willow shrubs, principally *Salix phylicifolia* and *S. commutata*, and occasional Pinaceae
3. A 50- to 70-year-old phase similar to phase 2 and showing denser vegetation
4. A 70- to 100-year-old phase, characterized by species of Cyperaceae, Ericaceae, Juncaceae, Onagraceae, Saxifragaceae, Scrophulariaceae and Pinaceae (*Abies lasiocarpa*, *Larix lyallii*, *Tsuga mertensiana*). These phases are referred to here as 20, 40, 60 and 80 years before present, respectively.

Sampling

Two chronosequence transects (eastern and western side) were established on the glacier forefront starting in the 20-year phase, where the transects were about 50 m apart, and ending in the 80-year phase, where the transects were about 200 m apart. Samples of barren (non-vegetated) soil were collected from the top 0–2 cm at five random locations for each phase in September 1994 and 1995, and pooled to make one sample per transect × phase combination. In 1995, soils under the canopies of non-mycorrhizal *Saxifraga ferruginea* and mycorrhizal *Salix* spp. were also collected, by digging up plant individuals of *Saxifraga* and shaking

the soil off the roots, and by collecting soils to a depth of 1–3 cm under the *Salix* shrubs. In September 1996, the barren soil of the 80-year phase was sampled again to re-check the 1994 respirometric results. Samples were initially kept on ice and deep-frozen within 4 days after sampling, except those subsamples from which the direct estimates of fungal and bacterial biomasses were made. All samples were sieved to 4 mm and organic matter (OM) determined by loss on ignition at 485°C for 4 h prior to microbial analyses.

1994 samples

Analyses of microbial biomass (active and total fungi and bacteria) were performed by direct estimates (microscopic counts) in E. Ingham's laboratory in Corvallis, Oregon. Active fungal biomass was measured by using the fluorescein diacetate (FDA) method (Ingham and Klein 1984) after weighing 1.00 g of fresh soil, shaking for 5 min in sterile water, properly diluting, staining with FDA for 3 min, adding 1.5% molten agar and placing an aliquot of the suspension on a slide with a well of known depth. Using an epi-fluorescent microscope (total magnification 250×), the length and diameter of active hyphae were measured from three transects on the slide. These were used to calculate the biovolume, which was converted to active biomass (ActF) using a density average of 0.41 g cm^{-3} (Van Veen and Paul 1979). Total fungal biomass (TotF) was measured using differential interference microscopy (total magnification 250×) from the same samples used for ActF and converted to biomass as for ActF.

The total number of active bacteria (i.e. FDA stained) was counted from the same slides by using epi-fluorescent microscope with a total magnification of 1000×. The average sizes and numbers of bacteria on the counted fields were used to calculate the biovolume, which was converted to active biomass (ActB) by using a density average of 0.33 g cm^{-3} (Van Veen and Paul 1979; Paul and Clark 1990). The total number of bacteria (dormant, senescent and active) and their average size were measured using the fluorescein isothiocyanate (FITC) method (Babiuk and Paul 1970) after weighing 1.00 g of fresh soil, shaking for 5 min in pH 7.2 0.2 M phosphate buffer, properly diluting, staining with FITC, and filtering the suspension on a non-fluorescent (iraglan-black stained) polycarbonate, 0.2 μm pore size filter. The size and number of fluorescent bacteria in ten fields per slide was counted using an epi-fluorescent microscope (400–425 nm exciter wavelengths, 490–510 nm barrier filter, total magnification of 1000×) and used to calculate the biovolume, which was converted to biomass (TotB) as described for ActB. Microbial biomass was calculated as a sum of ActB and ActF and converted to biomass C (C_{mic}) using a conversion factor of 0.5 (Van Veen et al. 1984).

From the same soil samples, microbial respiration was analysed as basal respiration and microbial biomass using the substrate-induced respiration (SIR) technique (Nordgren 1988; Palmborg and Nordgren 1993, 1996). The procedure, using a respirometer, gives estimates of the microbial biomass and activity, as well as their efficiency in exploiting added substrate. Respirometric analysis, i.e. analysis of evolved CO_2 from the soil samples as described by Nordgren (1988), included (1) the basal respiration rate (Bas) for a 30–35 h period, (2) SIR after addition of 200 mg of glucose, 22 mg of N as $(NH_4)_2SO_4$ and 2.4 mg of P as KH_2PO_4 per 100 g of soil sample, (3) time delay before exponential growth of microorganisms after substrate addition (Lag) and (4) specific growth rate (μCO_2). Previously untested variables were (5) maximum respiration (Max) after substrate addition, and (6) accumulated CO_2 (Acc) from the period beginning from substrate addition until Max was reached.

Max and Acc are variables that have not previously been used in research on microbial ecology. In this study, they were used as indices of the C use efficiency of the soil microbial community. Max and Acc would be expected to reach higher values when the microbial community is dominated by "energy-wasters" and functioning inefficiently, and lower values when the community is dominated by "energy-savers".

C_{mic} was calculated from SIR according to Anderson and Domsch (1978). Specific activity variables were calculated as ratios of Bas, Max and Acc to C_{mic} (i.e. qCO_2, qMax and qAcc, respectively), as estimated from the measurements.

1995 samples

Phospholipid fatty acid (PLFA) patterns were determined by extracting and analysing each soil sample as described by Pennanen et al. (1996). Briefly, 0.5 g fresh weight of soil was extracted with a chloroform:methanol:citrate buffer mixture (1:2:0.8) and the lipids separated into neutral lipids, glycolipids and phospholipids on a silicic acid column. The phospholipids were subjected to a mild alkaline methanolysis, and the fatty acid methyl esters were detected by gas-chromatography (flame ionisation detector) using a 50 m HP-5 (phenylmethyl silicone) capillary column and helium as a carrier gas. Peak areas were quantified by adding methyl nonadecanoate fatty acid (19:0) as an internal standard.

Total microbial biomass (TotPLFA) was determined as the sum of all the extracted PLFAs. The sum of PLFAs considered to be predominantly of bacterial origin (i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, 17:0, cy17:0, 18:1ω7 and cy19:0) was used as an index of the bacterial biomass (BactPLFA) (Frostegård and Bååth 1996). The quantity of 18:2ω6.9 was used as an indicator of fungal biomass (FungPLFA), since 18:2ω6.9 in soil is known to be of mainly fungal origin (Federle 1986) and it is known to correlate with the amount of ergosterol (Frostegård and Bååth 1996), a compound found only in fungi. The ratio of FungPLFA/BactPLFA was used as an index of the ratio of fungal/bacterial biomass in soil. The PLFAs i14:0, a15:0, i16:0 and 10Me18:0 are predominantly found in gram-positive (G^+) bacteria whereas the PLFAs cy17:0, cy19:0, 16:1ω7c and 18:1ω7 characterise gram-negative (G^-) bacteria. By independently summarising these PLFAs the G^+/G^- ratio was calculated.

Data analysis

Means and standard errors for each measurement were calculated using each transect as a replicate ($n = 2$). For the 1994 data the non-parametric Kruskall-Wallis test was used to compare the microbial variables across the different age phases. Two-way ANOVA was used for 1995 data, with age phase and soil sampling type (barren soil and soil under the canopies of *Saxifraga* or *Salix*) as independent variables. PLFA patterns were described by using the mol% of the individual PLFAs in canonical correspondence analysis (CA). Data analysis was performed using SAS programs (SAS 1988a,1988b), except CA for which a PC-ORD program (McCune and Mefford 1995) was used.

Results

Biomasses

Because of the small sample size ($n = 2$), our tests appear insensitive and the results should be considered with some caution. However, most results show clear trends and these tendencies will be discussed in order to formulate clearer hypotheses.

Both in the 1994 and 1995 samples, the OM of the soil tended to increase with increasing age from the glacial exposure, with the exception of the 80-year phase (Table 1). In the 1995 samples the OM in soils under the plant canopies tended to increase more than in barren soil (Table 1). In 1994 samples, ActF and ActB tended to increase along the transects 8- and 3- to 4-fold,

respectively (Table 2), while accumulation of OM was 2.5-fold, indicating that organic matter became more densely inhabited by microorganisms with increasing successional development of the plant community. The ratio of ActF to ActB increased from 2 to 7 and the ratio of TotF to TotB from 0.2 to 12, indicating that the microbial community shifted to a fungal-dominated one. TotB tended to decrease through the transect. The ratio of ActB to TotB was 0.02 in the very early successional stages but later seemed to stabilize to about 0.10 (Table 2). Fungal biomass accumulated, as shown by an increase in TotF, which was 30 times greater in the 80-year phase than in 20-year phase; over this period ActF/TotF tended to decrease (Table 2). C_{mic} calculated from the SIR values increased slightly over the successional gradient (Table 2).

TotPLFA of the barren soil tended to increase with time since glacial retreat, but this trend was not observed in the soil collected under the two plant species and the microbial biomass instead remained constant from the 20- to the 60-year phase and declined by the 80-year phase (Fig. 1A).

Activities

Concomitant with the increasing biomasses, Bas increased 2.5-fold from the 20- to the 80-year phase (Table 3). The other activity variables, Lag and μCO_2, did not show any clear relationship with successional time. Lag was clearly lower at the 80-year phase than at the 20- to 60-year phases (Table 3), but re-measurement in 1996 failed to confirm that the soil from the 80-year phase differed greatly from the others. Max and Acc showed declines along the gradient (Table 3). The specific activities (qCO_2, qMax, qAcc) which were calculated per unit biomass from direct estimates tended to decrease sharply with successional development, but when calculated per biomass from SIR values, the trends were not as clear. qMax and qAcc

Table 1 Organic matter (OM) content (mg g^{-1} dry weight, d.w.) in the barren soil in 1994 and in the barren soils and in the soils under the *Saxifraga* and *Salix* canopies in 1995. Mean and SE ($n=2$)

Age of the phase (years)	Barren soil 1994		Barren soil 1995		Soil under *Saxifraga* canopy 1995		Soil under *Salix* canopy 1995	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
20	2.3	0.5	3.3	0.5	5.4	0.7	–	–
40	3.9	0.4	5.0	0.3	8.7	1.5	13.3	2.7
60	3.3	0.5	6.1	1.0	11.7	3.0	22.1	3.8
80	5.7	1.0	5.5	0.8	10.5	3.2	15.4	0.8

Table 2 Analysis of microbial biomass (mean and SE) in barren soil in 1994. Active and total fungal and bacterial biomasses (*ActF*, *TotF*, *ActB* and *TotB*, respectively) are expressed on an OM basis. Biomass C (C_{mic}) is calculated from substrate-induced respiration (SIR) values (Anderson and Domsch 1978). χ^2 is the chi-square approximation for the difference among the phase ages according to the Kruskal-Wallis test ($n=2$)

Age of the phase (years)	ActF (mg g^{-1} OM)		ActB (mg g^{-1} OM)		ActF/ActB	
	Mean	SE	Mean	SE	Mean	SE
20	0.93	0.24	0.46	0.09	2.0	0.1
40	2.18	1.56	1.26	0.17	1.6	1.0
60	5.22	0.05	1.92	0.77	3.2	1.3
80	7.62	1.59	1.24	0.38	7.3	3.5
χ^2	6.00		4.50		3.67	
P	0.112		0.212		0.300	
	TotF (mg g^{-1} OM)		TotB (mg g^{-1} OM)		TotF/TotB	
	Mean	SE	Mean	SE	Mean	SE
20	3.3	0.8	20.9	4.1	0.2	0.1
40	11.1	8.3	12.3	1.1	0.8	0.6
60	26.4	12.4	14.5	2.3	2.0	1.2
80	97.2	4.0	8.4	1.3	11.8	1.4
χ^2	5.50		6.17		6.17	
P	0.137		0.104		0.104	
	ActF/TotF		ActB/TotB		C_{mic} (mg C g^{-1} OM)	
	Mean	SE	Mean	SE	Mean	SE
20	0.28	0.00	0.02	0.01	4.8	0.6
40	0.21	0.02	0.10	0.00	5.5	1.3
60	0.26	0.12	0.13	0.03	7.3	0.2
80	0.08	0.02	0.14	0.02	7.4	0.1
χ^2	4.67		5.17		5.50	
P	0.198		0.160		0.139	

decreased over time but qCO_2 showed a slight increase (Table 4).

Community structure

The barren soil had the lowest TotPLFA values, while the soil under *Saxifraga* had the highest (Fig. 1, Table 5). The same trend was also observed for BactPLFA and FungPLFA along the gradient. The soil type, but not the soil age, had a significant effect (Fig. 1B, C, Table 5). The changes that had occurred by the 80-year phase for all three sample types were due to shifts in the fungal component of the microbial biomass, as shown by the fungal/bacterial biomass ratio (Fig. 1D). The fungal biomass increased in the barren soil and decreased under the plant canopies at the 80-year phase. Soil under the mycorrhizal plants (*Salix*) seemed to have higher fungal/bacterial biomass ratios than under non-mycorrhizal plants (*Saxifraga*), indicating the presence of mycorrhizal fungi. The G^+/G^- ratio was higher under the canopies than in the barren soil (Fig. 1E, Table 5).

CA separated the three soil types from each other: barren soil samples showed high variation in their PLFA patterns but the soil samples under the plant canopies were grouped close to each other (Fig. 2). Barren soil samples from the oldest phase also were grouped close to the plant canopy samples. No trend with time was found.

Indicative PLFAs that separated the barren soil samples from the 20- to 60-year phases from the vegetated soils were 20:4, 17:0 and 10Me16, which were less abundant in the soils under plant canopies, and 19:1b, 19:1a, i14:0 14:0, 16:1ω7t, 18:2ω6, 10Me18 and C:18, which were more abundant in the soils under plant canopies (Fig. 2). This grouping pattern could also be achieved using the bacterial PLFAs by removing the fungal PLFA 18:2ω6 from the data set, indicating that the rhizosphere effect in soils close to plant roots also affected the bacterial community.

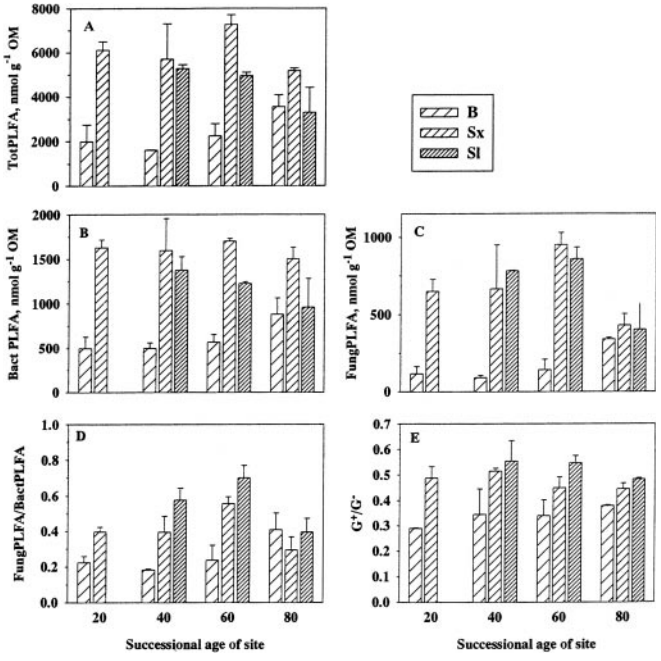

Fig. 1 **A** Total, **B** bacterial and **C** fungal PLFA concentrations, ratios of **D** fungi to bacteria and **E** gram positive to gram negative bacteria (*B* barren soil, *Sx* soil under *Saxifraga ferruginea* canopy, *Sl* soil under *Salix* spp. canopy). *Vertical bars* represent SEs for $n = 2$

Discussion

Microbial biomass showed an increase across the gradient regardless of the method used. The increase in biomass was seen most clearly using direct estimates, which also supports the results for FungPLFA in barren soil samples. A problem with microbial biomass analyses is that most methods measure only subset components of the total microbial biomass or do not treat the active and dormant components equivalently (Wardle and Parkinson 1991; Ohtonen 1994). Thus the results using different methods are not necessarily comparable, as was seen here. Biomass calculated from SIR values was 2–8 times greater than the sum of active fungi and bacteria from direct estimates. Direct estimates and PLFA measurements both showed that the ratio of fungi to bacteria increased over time, although these two methods are still

Table 3 Respirometric analysis of barren soil in 1994. Basal respiration (*Bas*), maximal respiration after substrate addition (*Max*) and accumulated CO_2 at the moment of maximum respiration (*Acc*) are expressed on OM basis. *Lag* is the time from substrate addition to the start of exponential growth of microorganisms and μCO_2 is the specific growth rate. χ^2 is the chi-square approximation for the difference among the phase ages according to the Kruskal-Wallis test ($n = 2$)

Age of the phase (years)	Bas (mg CO_2-C g^{-1} OM h^{-1})		Lag (h)		μCO_2		Max (mg CO_2-C g^{-1} OM h^{-1})		Acc (mg CO_2-C g^{-1} OM h^{-1})	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
20	0.011	0.002	27	17	0.026	0.002	14.3	0.6	104	17
40	0.013	0.002	21	4	0.036	0.001	10.1	0.7	52	6
60	0.020	0.002	32	13	0.032	0.003	9.0	0.7	55	6
80	0.028	0.004	9	4	0.032	0.002	8.1	1.8	43	6
$\chi 2$	6.17		3.50		4.50		4.50		4.67	
P	0.104		0.321		0.212		0.212		0.198	

Table 4 Specific activities (mean and SE) of the microbial community in the barren soil in 1994. Measurements are specific activity (qCO_2), specific maximal activity ($qMax$) and specific CO_2 accumulation ($qAcc$) calculated using two independent biomass estimations. χ^2 is the chi-square approximation for the difference among the site ages according to the Kruskal-Wallis test ($n = 2$)

Biomass method	Age of the phase (year)	qCO_2 (g CO_2-C $g^{-1}C_{mic}$ h^{-1})		qMax (g CO_2-C $g^{-1}C_{mic}$ h^{-1})		qAcc (g CO_2-C $g^{-1}C_{mic}$)	
		Mean	SE	Mean	SE	Mean	SE
Direct estimates	20	0.0174	0.0066	22.1	6.1	164	63
	40	0.0111	0.0069	7.6	3.4	38	16
	60	0.0057	0.0013	2.5	0.1	15	0
	80	0.0066	0.0017	1.9	0.7	10	3
	χ^2	3.00		6.17		6.67	
	P	0.392		0.104		0.083	
SIR	20	0.0024	0.0000	3.1	0.3	21.7	0.51
	40	0.0029	0.0015	1.9	0.3	9.8	1.3
	60	0.0033	0.0003	1.2	0.1	7.6	1.0
	80	0.0048	0.0015	1.1	0.3	5.9	1.0
	χ^2	2.83		6.00		5.50	
	P	0.418		0.112		0.139	

Table 5 Results (F values and significance) of two-way ANOVAs testing effects of the vegetation phase class (*Age*) and soil type (barren soil and soils under the canopies of *Saxifraga* and *Salix*), for the total (*Tot*), bacterial (*Bact*) and fungal (*Fung*) phospholipid fatty acid (*PLFA*) content ($n = 2$) (G^+/G^- gram-poisitive to gram-negative bacterial ratio)

	df	TotPLFA		BactPLFA		FungPLFA		FungPLFA/BactPLFA		G^+/G^-	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Model	10	7.05	0.0017	7.12	0.0016	6.85	0.0019	5.98	0.0033	3.43	0.0274
Age	3	0.77	0.5321	0.11	0.9522	2.51	0.1123	2.45	0.1182	0.41	0.7460
Soil type	2	28.31	0.0001	32.11	0.0001	22.62	0.0001	15.25	0.0007	13.73	0.0010
Age × Soil type	5	2.29	0.1173	1.29	0.3361	2.76	0.0745	3.64	0.0346	0.66	0.6625

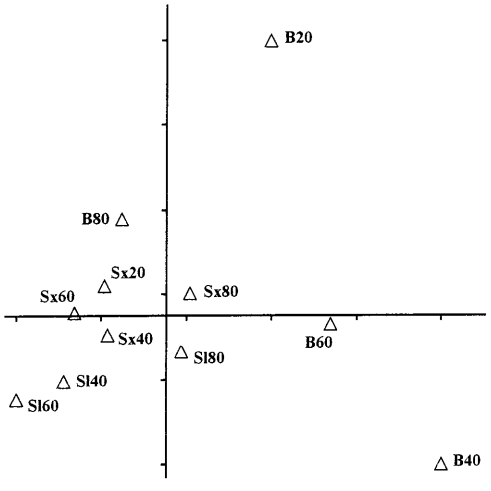

Fig. 2 Canonical correspondence analysis (CA) of the PLFA data. *Sample codes* refer to the soil type and phase on the gradient (20- to 80-year) (*B* barren soil, *Sx* soil under *Saxifraga ferruginea* canopy, *Sl* soil under *Salix* spp. canopy)

not directly comparable. From the PLFA analysis several indicative PLFAs for bacteria but only one for fungi can be identified, which may lead to a misleading interpretation of the real fungi/bacteria ratio.

It is well known that plant rhizospheres attract soil microorganisms (Paul and Clark 1989) and this might be why higher biomass was observed in soil under *Salix* and *Saxifraga* during the early years of succession. According to pure culture isolation techniques the plant rhizospheres have been found to be characterized by a higher proportion of gram-negative bacteria (Paul and Clark 1989). We found the opposite in the field: the G^+/G^- ratio was higher under the plant canopies than in the barren soil. The G^+/G^- ratio of the barren soil increased through the transect but little change was observed for the soils under canopies over time.

The development of the FungPLFA/BactPLFA and the G^+/G^- ratios of the barren soil of the 80-year phase towards levels which characterize soils under plant canopies indicates that the barren soil at that stage is interwoven with fine roots and it is actually the rhizosphere effect that is being measured. The extension of the root system affects the surrounding soil biota. *Saxifraga ferruginea* may have a very extensive root system even at the last rosette stage, reaching 10 cm deep and almost as wide (authors, personal observations). As a very tender and small-leaved plant, *Saxifraga* does not accumulate litter at its base in the way that *Salix* shrubs do. Thus roots (root litter and exudates) rather than leaf litter are mainly responsible for

greater microbial biomass being present under plant canopies than in barren soil. This would drive microbial biomass build-up in soil even when there are few above-ground plant parts but where an expanding root system exists.

The 80-year phase is located on the south slope of a rather tall terminal moraine, and Jumpponen et al. (1998) previously noticed the decrease in soil OM and N concentration under willow canopies in the same location. The reason for this unexpected result may be due to leaching and wind or water erosion resulting from aspect (Jumpponen et al. 1998). Moreover, as TotPLFA values decreased in the rhizosphere soils but not in the barren soil samples, another explanation could be the increased competition between plants and soil microorganisms for nutrients with increasing successional age. Vegetation is more dense at the 80- than at the 60-year phase, and this may cause more intensive competition for scarce N and other nutrients, thus inducing a decrease in the soil microbial biomass. As the degree and nature of competition between plants and soil microorganisms still remain unclear, more research is needed before the nature of N limitation in terrestrial ecosystems can be fully understood (Kaye and Hart 1997).

Microbial activity parameters were somewhat lower but Lag longer than is usual for boreal forest soils (Palmborg and Nordgren 1993; Ohtonen 1994; Väre et al. 1996; Merilä and Ohtonen 1997; Palmborg et al. 1998). The general range for Bas in forest soils has been found to be 0.01–0.05 mg CO_2-C g^{-1} OM h^{-1} and that for Lag about 10–20 h This may indicate harsh conditions in early succession and slow metabolism of microorganisms. For Bas this may certainly be true but it appears more likely that Lag reflects slower diffusion rates of added substrates in quite large samples of mineral soil (about 100 g fresh weight, f.w.) compared to the much smaller humus samples (about 5 g f.w.) used in the analysis of forest soils. We thus suspect that Lag does not have any indicative value when describing the characteristics and function of the microbial community in the soils of various ages in this succession. However, Lag has been found to increase markedly in soils polluted with heavy metals (Nordgren et al. 1988; Palmborg and Nordgren 1996) and due to disturbance (decrease in vegetation and soil OM) caused by reindeer grazing (Väre et al. 1996; Ohtonen and Väre 1998), indicating other kinds of changes in soils than just successional processes.

The new activity variables proposed here, Acc and Max, do not depend on the diffusion rate of added substrate because these measurements are independent of time. High Max suggests that the microbial community cannot incorporate all the added substrate into biomass, but is capable of quickly increasing the rate of respiration, resulting in extra C being lost as CO_2. Large amounts of respired C also result in large levels of total accumulated CO_2 and thus high values of Acc. However, high Acc values do not necessarily mean high respiration rates on an hourly basis.

Metabolic rate constants such as qCO_2 are normally used in pure culture studies to describe growth or nutrient use characteristics of the micro-organisms (Anderson 1994). In nature, different species compete for the same substrate, which will certainly affect the metabolism of the individual members of the community as well as total community metabolism. Competition for substrates and C-limiting conditions are controlling factors of metabolic rate constants which can be used to describe the metabolism of complex microbial communities (Anderson 1994). In our study, the microbial community at the later successional stage could not reach as a high respiration rate per unit biomass as the early-stage microbial community but was living in an "energy-saving" state. Although this was apparent from the qCO_2 and qMax data, it was most apparent for the qAcc data, and we therefore suggest that qAcc performs as a better indicator than qCO_2 with regard to the energy use efficiency of the microbial community, especially given that qCO_2 yields confusing interpretation (Wardle and Ghani 1995).

The values for specific activities were greatly dependent on the method used for the biomass estimation. The general trend was that the microbial community shifted from an energy-inefficient one towards an energy-efficient one with increasing age of soil. The slow increase in microbial activities relative to that of biomass in response to successional age is likely to be due to the allocation of C to microbial biomass maintenance rather than to respiration, which is in accordance with results from other studies of primary succession (Insam and Haselwandter 1989; Anderson 1994; Wardle and Ghani 1995). The decrease in specific activities can also be connected with alterations in the fungal to bacterial ratio. Fungi are more effective in their energy use than bacteria, and also build up a large inactive biomass as was seen from the direct estimates. Thus with an increasing proportion of fungi in the soil, the energy use efficiency increases resulting in decreased specific activities. This interpretation differs from that of Insam and Haselwandter (1989) and points to the need to study microbial community structure in conjunction with activity analyses, as proposed by Wardle and Ghani (1995) and Ohtonen et al. (1997).

In conclusion, the evidence supported our hypotheses, i.e. that C use efficiency of the micro-organisms increases with successional age, that changes in the C use efficiency are related to the microbial community structure assessed by both bacterial/fungal ratios and by PLFA analyses, and that the rhizosphere modifies the microbial community structure.

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