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Long-term manipulation of the microbes and microfauna of two subarctic heaths by addition of fungicide, bactericide, carbon and fertilizer

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Abstract

Nutrient availability is a major constraint to plant production and carbon storage in arctic ecosystems, but there are few studies coupling processes in the decomposer and microbial food web and the implications these processes have on the control of nutrient mineralization. We studied the relationship between microbial biomass and the abundance of microbivore and the role of grazing on nutrient turnover after annual addition of carbon (sucrose), fertilizer (NPK), fungicide (benomyl) and bactericides (streptomycin and penicillin) to two dwarf shrub communities, a low and a high altitude heath. After four years of repeated additions, we measured microbial biomass by fumigation-extraction and phospholipid fatty acid (PLFA) analysis, the fungal to bacterial biomass ratio by PLFA analysis and estimated the numbers of protozoa and nematodes, assigned into feeding groups. The fungal to bacterial ratio of nematode feeding groups was around 0.2, indicating a bacterial-based food web in both communities. The size of the microbial biomass did not change after the additions, except when the amount of available carbon was increased (low altitude heath) or when addition of carbon was combined with fertilizer (high altitude heath). In contrast, fertilizer but not carbon increased the number of microbivores. This suggests that the amount of available carbon and not grazing pressure controls the size of the microbial biomass. Furthermore, it suggests that the food quality, e.g. nutrient content of the micro-organisms, had a larger effect on the microbivore than the size of the microbial biomass. The addition of bactericides and fungicide did not significantly change the fungal to bacterial biomass ratio of the micro-organisms. We could not detect any effects of the bactericides. In contrast, the fungicide strongly decreased nematode density, least in the fungal feeders, probably due to increased abundance of the insensitive *Aphelenchoides* ssp.

Keywords: Microbial biomass; Phospholipid fatty acids; Nematode feeding groups; Soil food web; Protozoa

1. Introduction

Nitrogen and phosphorus availability are major constraints to plant production and carbon storage in the Arctic (e.g. Shaver and Chapin, 1995) and are the major factors influencing ecosystem carbon storage. Micro-organisms are both a source and a sink of nutrients due to the opposing processes of mineralization and nutrient immobilization. Inorganic nutrients may be released directly by mineralization activities of micro-organisms or indirectly through grazing on the

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microbial biomass by microbivores. The microfauna, such as protozoa and nematodes, constitute a larger part of the total faunal biomass and activity in arctic soils than in, for example, temperate soils, due to the absence or low number of larger invertebrates in arctic soils (Ryan, 1981; Heal, 1997; Sohlenius et al., 1997). Microfaunal grazing on primary decomposers, bacteria and fungi, may therefore exert a major effect on the energy and nutrient flow through the soil system.

The most intensive large-scale study of arctic soils, the International Biological Programme in the 1970s, included only few investigations of the microfauna, but available data showed high numbers of protozoa and nematodes (Lagerlöf et al., 1975; Svensson and Rosswall, 1980). Lagerlöf et al. (1975) found that nematodes constituted 30% of the faunal biomass at a mire site. The recycling of labile nutrients is promoted by microbial consumption of the microfauna (Clarholm, 1985, 1989; Kuikman and van Veen, 1989), but the relative importance of grazing on nutrient mobilization in arctic soils is unknown. Grazing on microbes by nematodes and protozoa may enhance the rate of decomposition and mineralization, as observed outside the arctic (Anderson et al., 1983; Clarholm, 1985; Freckman, 1988; Beare et al., 1992; Rutherford and Juma, 1992; Ferris et al., 1997).

In order to study the relationship between microbial nutrient immobilization and nutrient availability in arctic heath soils, we initiated an experiment with manipulations of the microbial access to nutrients (NPK) and energy (carbon). Furthermore, we attempted to manipulate the fungal to bacterial ratios by selective inhibition of the fungal and bacterial communities through application of a fungicide (benomyl) and two bactericides (penicillin and streptomycin).

After one growing season, we found increased microbial biomass C after carbon addition without changes in the microbial N and P pool sizes (Jonasson et al., 1996). Addition of NPK caused increases in microbial N and P, which was further increased if the NPK addition was combined with addition of labile C (sucrose) (Jonasson et al., 1996), as observed in several other short term experiments (Schmidt et al., 1997a,b). However, there was a low response in microbial C to temperature enhancement and NPK addition at two different, nearby heath types after five years of manipulations (Jonasson et al., 1999). In contrast, nematode density increased in response to these treatments (Ruess et al., 1999) indicating that the microbial productivity may have increased. This was supported by increased net mineralization and N and P plant pool sizes in the warmed plots.

Nematodes are indicative of changes in the decomposition food web, because they play an important role in the fungal, bacterial as well as root energy channels, as consumers of plant roots, bacteria and fungi (Freckman, 1988; Freckman and Ettema, 1993; Mikola and Setälä, 1999). Further, models indicate that changes in net primary production would cause changes in nematode densities that reflect the rates of material flow through the system (Moore and de Ruiter, 1993). The largest contribution to the faunal biomass in arctic soils is assumed to be the protozoa (Svensson and Rosswall, 1980), but the abundance and function of this group is barely studied in arctic ecosystems.

In this paper we investigate the long-term responses in soil microbial biomass and protozoa and nematode numbers to four years of repeated applications of carbon, fertilizer, fungicide and bactericide at two heaths. Firstly, we measured the size of the microbial biomass with two different methods, viz. chloroform-fumigation-extraction (CFE) and phospholipid fatty acid (PLFA) analysis. We estimated the density of protozoa by the most probable number method (Darbyshire et al., 1974; Rønn et al., 1995) and counted nematodes after modified Baermann extraction (see Ruess, 1995). We hypothesized that the long-term response of microbial biomass C to nutrient and carbon amendment would be small, but that there would be an increase in nematode and protozoa indicating increased microbial productivity.

Secondly, we identified the PLFAs and their relative distribution in order to get a rough estimate of the response in fungal to bacterial (f/b) biomass ratios. Moreover, the nematodes were assigned to feeding groups so that changes in nematodes could be related to changes in microbial community. We expected to find decreased f/b biomass and feeding group ratios in micro-organisms and nematodes after application of fungicide and increased f/b biomass and feeding group ratios after application of bactericides and decreased protozoa.

2. Materials and methods

2.1. Study sites

The study took place in two dry to mesic dwarf shrub communities, a low and a high altitude heath near Abisko Scientific Research Station in North Sweden. The low altitude heath site is located close to Mount Paddusstieva at the tree line at 450-m a.s.l. and the high altitude site is at Mount Slåttatjåkka at 1150-m a.s.l. The climate is subarctic, with a growing season of approximately 3 months. The mean annual air temperature and precipitation recorded at the nearby research station are −0.5°C and 299 mm, respectively. In the 1996 growing season, when the measurement took place, the mean air temperature was 10.2°C at the Research Station with mean soil temperatures at 5

and 2 cm depth around 7.5 and 6.5°C at the low and high altitude heath, respectively. The low altitude heath soil has an organic layer of 10–15 cm depth and a pH_{H_2O} around 7.1, whereas the high altitude site has a soil organic layer of approximately 2 cm depth and pH_{H_2O} around 5.2 (Table 1). The low altitude site has closed dwarf shrub vegetation with a dense moss cover, whereas the high altitude site has open dwarf shrub vegetation with a discontinuous moss layer. For further characteristics of soil and vegetation, see Table 1 and Jonasson et al. (1996).

2.2. Experimental design and perturbations

The two dwarf shrub communities have been subjected to factorial application of fertilizer (5:1.25:3.75 g m^{-2} NPK), carbohydrate (250 g commercial sugar (sucrose) m^{-2}) and fungicide (4 g Benlate with 2 g of the active component benomyl m^{-2}) since 1993 (Table 1). Six blocks with eight 1 × 1 m squares were subjected to the manipulations just after snowmelt in mid to late June and again in mid-summer in July. In 1994, the experiment was extended with two additional treatments: bactericide (2 g streptomycin and 2 g penicillin m^{-2}) and a combined fungicide and bactericide treatment. All additions were applied as water solutions (2 l m^{-2}) except for sugar, which was applied as fine granulates. We applied the same amount per m^2 at both sites, but due to large differences in the depth of the organic layer, the additions per g soil organic matter were almost three times higher at the high altitude heath. In 1996, the year of sampling, fertilizer, sugar, fungicide and bactericides were applied 27 June and 18 July at the low altitude heath and 28 June and 20 July at the high altitude site.

Table 1
Selected properties of the organic layer of the two experimental sites, a low and a high altitude heath. Data are based on measurements in the control plots ($n = 6$) in late August 1996 in the upper 10 cm of the organic layer in the low altitude heath, whereas the measurements are based on the entire organic layer at the high altitude site. SOM (soil organic matter), DOC (dissolved organic carbon)

	Low altitude heath	High altitude heath
pH_{H_2O}	7.1	5.2
Depth of SOM (cm)	15	2
% loss on ignition (SOM)	81	41
SOM (g m^{-2})	6900	2600
DOC (g m^{-2})	14.8	3.9
Total soil N (g m^{-2})	159	51
Total soil P (g m^{-2})	8.2	6.6
Soil C:N ratio	21.9	25.9
H_2O % of DW	222	169
Additions June and July		
C in 250 g sugar m^{-2}	100	100
N in NPK fertilizer (g m^{-2})	5	5
P in NPK fertilizer (g m^{-2})	1.25	1.25
K in NPK fertilizer (g m^{-2})	3.75	3.75
Benomyl (g m^{-2})	2	2
Penicillin (g m^{-2})	2	2
Streptomycin (g m^{-2})	2	2

2.3. Soils and sampling

Soil samples from the organic layer were collected 20 and 22 August at the low and high altitude site, respectively. Three soil cores (⌀ 4.5 cm) of 10 cm depth were collected from the organic layer at the low altitude heath, whereas 3–6 cores were collected from the shallow organic layer at the high altitude site to a total length of 15 cm organic soil per plot. The cores from each plot were pooled, mixed and sieved within 24 h and used for analyses of microbial biomass C, pH, water content and SOM measured as loss on ignition at 550°C. The rest of the soil (100–200 g) was divided into two samples. One subsample was kept at 2°C for a month before analysis of protozoa. Another subsample was frozen for later analysis of PLFA. Soil samples for nematodes were taken with a soil corer (⌀ 2.5 cm) from each treatment plot and divided into two horizons of 0–3 and 3–6 cm depth at the low altitude heath and 0–2 cm depth at the high altitude site. Samples were stored (maximum 48 h) at 2°C until extraction of the nematodes.

2.4. Microbial biomass C and phospholipid fatty acid fractions

In the laboratory, sieved soil from each sample was extracted in 0.5 M K_2SO_4 (1:5 w:v) for 1 h. Starting at the same time as the extraction, another subsample was fumigated with chloroform for 24 h to release the microbial biomass C (Jenkinson and Powlson, 1976; Tate et al., 1988) followed by extraction.

Dissolved organic carbon (DOC) was analyzed in the extracts using a Shimadzu Total Organic Analyzer, TOC 5000A. The microbial C content was estimated as the difference in carbon content of the extracts of the unfumigated and the fumigated soil sample. To convert the amounts of microbial C released by chloroform fumigation and extraction (CFE) to total microbial biomass C, we assume an extractability for C of 0.45 (Wu et al., 1990; Martens, 1995; Joergensen, 1996).

PLFAs were extracted and analyzed according to the method of Frostegård et al. (1991); Frostegård and Bååth (1996) on five of the six replicate blocks sampled at the low altitude heath and four blocks at the high altitude site. From the previous frozen subsample, 0.6 g sieved soil were extracted in one-phase chloroform:-methanol:citrate buffer, split into two phases by adding chloroform and citrate buffer and fractionated into lipid fractions on silicic acid columns. The polar lipids,

which contain the phospholipids, were dried and transformed into free fatty acid methyl esters by alkaline methanolysis and measured on a gas chromatograph with flame ionization detector. Nonadecanoate (fatty acid 19:0) was added as an internal standard before methanolysis. The fatty acids were identified using the retention times on the chromatogram by comparing with the retention time of fatty acids earlier determined using GC–MS. A rough estimate of microbial biomass was calculated from total PLFA concentration using a conversion factor of 340 nmol PLFA mg^{-1} biomass C (Frostegård et al., 1991). An index of the fungal to bacterial ratio of the microbial biomass was calculated using PLFAs 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 for bacteria and 18:2ω6,9 for fungi (Frostegård and Bååth, 1996). The fungal:bacterial ratios were used to relate treatment changes in micro-organisms to changes in nematode trophic structure, but not as an absolute estimate of fungal and bacterial biomass.

2.5. Nematodes

Nematodes were extracted from the soil using a modified Baermann method (for details see Ruess, 1995). After 24 h extraction at 10–12°C, a heating regime was started using electric bulbs placed over the extraction funnels. Temperature was increased gradually and reached 32°C after 6 h. The extracted nematodes were preserved in a 4% formaldehyde solution. The individuals extracted from each sample were counted. Ten percent of the individuals from each sample (but not less than 100 per sample) were assigned to feeding groups according to Yeates et al. (1993). Altogether 163,900 nematodes were counted and 21,500 of them were determined with respect to feeding group. The ratio of fungal to bacterial feeders was calculated as f/b (Freckman and Ettema, 1993). An estimate of total nematode biomass C was calculated from the weight per individual within each feeding group as given by Sohlenius et al. (1997), taking the abundance of each group into account. We assumed that 25% of the nematode fresh mass was dry weight and had a carbon content of 50% in the dry mass.

2.6. Protozoa

Protozoan abundance in the low altitude heath soil was estimated by a most probable number (MPN) approach. Soil portions of 0.8 g were suspended in 16 ml modified Neff's amoebae saline (Page, 1988) in 116 ml serum bottles and shaken for 30 min on a 'Heidolph Promax 2020' shaker at 390 rpm. After shaking, the suspension was diluted 3-fold with sterile tryptic soy broth (100 mg l^{-1}, Difco BactoR) and mixed. From this dilution 11 more successive 3-fold dilutions were made similarly. Four ml from each of the 12 dilutions was used to inoculate a 96-well microtitre plate, 100 μl in each well and eight replicates of each dilution. Plates were stored at 12.5°C in darkness. Microtitre plates were inspected at day 7 and 21 by an inverted microscope for presence of protozoa. Information from the microtitre plates was transformed to most probable numbers of organisms as in Rønn et al. (1995). To estimate protozoan biomass, it was assumed that half of the protozoa were flagellates and half of them were naked amoebae and that heterotrophic flagellates and naked amoebae had individual biovolumes of 50 and 400 μm^3, respectively (Stout and Heal, 1967). The dry weight was assumed to be 0.2 pg μm^{-3} (Meisterfeld, 1989) and the carbon content to be 50% (Coleman et al., 1978).

2.7. Statistical analysis

All statistical analyses were performed with SAS (Statistical Analysis System Institute, 1990). Analysis of variance (ANOVA) was used for all data with type III sums of squares in the GLM procedure. Treatment effects on DOC, microbial C and PLFA per unit soil organic matter (SOM) were analyzed by three-way factorial ANOVAs with carbon, fertilizer and fungicide as main effects including all interactions. The data were log-transformed to meet the assumption of equality of variance in ANOVAs. Protozoa and nematodes were analyzed by non-parametric ANOVAs on ranked data sets testing the main effects without interactions. The treatments with bactericides and combined bactericides and fungicide were initiated a year later than the factorial design with carbon, fertilizer and fungicide and were not included in the ANOVAs. The means of the single treatments, including the bactericide treated soils, were tested using Dunnett's two-tailed *t*-test for differences from the control at the significance level of 0.05. All significant effects from the ANOVAs ($P < 0.05$) are given in Fig. 1 or 3.

To study changes within the microbial community, the molar percentage of individual phospholipid fatty acids (PLFAs) was subjected to principal component (PC) analyses, one for each site. The data were standardized to unit variance before the analyses.

3. Results

3.1. Microbial biomass C, DOC and fungal to bacterial biomass ratio

The concentrations of microbial biomass C measured by the CFE procedure were 5.9 and 11.0 mg

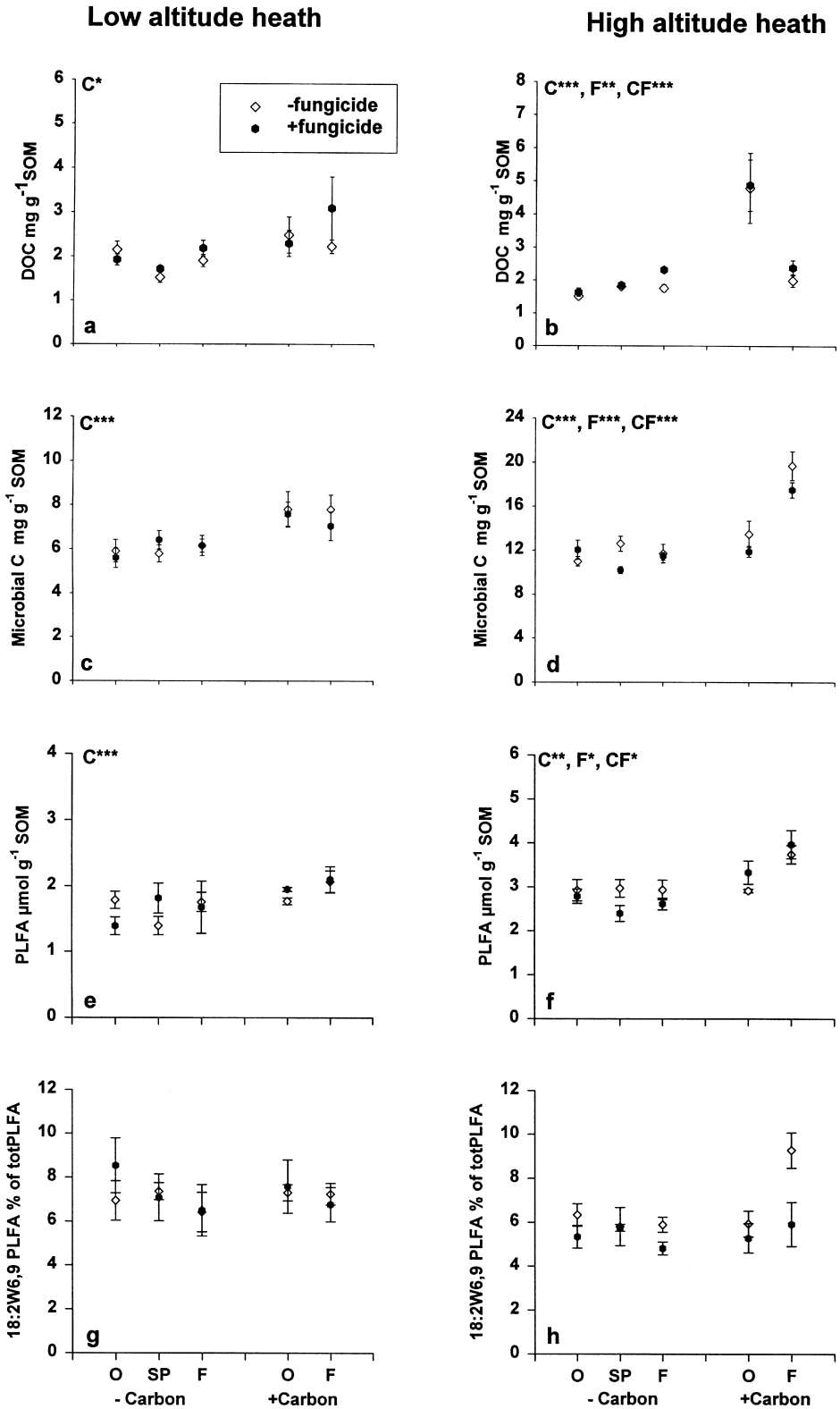

Fig. 1. DOC (a, b), microbial C (c, d), PLFA (e, f) and mol% of 18:2w6 (g, h) in August 1996 after four growing seasons with application of benomyl (B), carbon (C), bactericides (SP) and fertilizer (F) and in control plots (0) at a low and a high altitude heath (means $\pm$ S.E., $n = 6$). Results of ANOVAs in the factorial design with significant effects of the main factors benomyl (B), carbon (C) and fertilizer (F) and interactions are given at the top of the diagram. Levels of significance: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

g^{-1} SOM in the control plots of the low and high altitude heath, respectively (Table 2). Across all treatments at both sites, this microbial C estimate was significantly correlated with the biomass C estimate from the PLFA analysis with $r = 0.53$ and 0.68, for the low and high altitude sites, respectively ($P < 0.001$, Pearson correlation coefficient). The estimates of the PLFA analysis were approximately 70–80% of the CFE-estimates (Tables 3 and 4) using the conversion factors given in Section 2.

The amount of dissolved organic carbon (DOC) was approximately one third of the amounts of C in the microbial biomass at the low altitude heath (Fig. 1a). Carbon addition increased the DOC concentration significantly ($P < 0.05$ and $P < 0.001$ in the low and high altitude soil, respectively), one month after the application. The largest response was in the high altitude soil, where DOC in carbon treated plots was still three times the level of the control plots one month after the application. When the addition of carbon was combined with NPK fertilization at the high altitude site, there was a significant decrease in the concentration of DOC ($P < 0.001$) which declined to the same level as in the control.

At the low altitude heath, there was a significant effect of carbon addition on microbial biomass C and total amount of PLFA ($P < 0.001$; Fig. 1c and e). Application of fertilizer, bactericide or fungicide did not result in significant changes in the microbial C. At the high altitude heath, there was a significant effect of both fertilizer and carbon addition, but also a strong C × F interaction. Microbial C and PLFA increased only when the carbon addition was combined with fertilization on this site (Fig. 1d and f). A Dunnett's test did not reveal any significant effect of fertilizer or carbon additions alone.

In general, the proportion of the two eucaryotic PLFAs, 18:2ω6,9 and 18:1ω9, increased when the total amount of PLFA increased, except for 18:2ω6,9 after combined carbon and benomyl treatments (Fig. 1g and h). The two PLFAs accounted for at least 50% of the increase in microbial biomass after carbon addition at the high altitude heath. The index of fungal to bacterial biomass ratio estimated from PLFAs was 0.19 in the control plots at both sites (Table 2). At the low altitude heath, the abundance of the fungal component (18:2ω6,9) of the PLFAs did not respond significantly to the treatments with fungal to bacterial ratios ranging from 0.17 to 0.23 (Table 3 and Fig. 1g). In contrast, at the high altitude site, there was a significant main effect of benomyl addition on the fungal to bacterial ratio ($P < 0.01$; Table 4, Fig. 1h) with a decrease of the ratio to 0.15. In contrast, the combination of carbon and fertilizer addition increased the relative abundance of 18:2ω6,9 ($P < 0.05$, Dunnetts test, Fig. 1h) and strongly increased the f/b ratio (Table 4).

3.2. PLFA distribution

In the low altitude heath the PC analyses revealed a significant ($P < 0.01$) fertilizer effect on the microbial community along the first principal component, where fertilized plots were found to the right in the PC graph (Fig. 2a). The second component differed significantly ($P < 0.001$) between carbon treated plots, found in the lower half of the graph, and treatments without carbon addition, found in the upper half. The first component explained 25.5% and the second 19.4% of the total variation in the PLFA data.

Table 2
Selected biological parameters from the organic layer of the control plots ($n = 6$) at the two experimental sites, a low and a high altitude heath in late August 1996. Data represent the upper 10 cm of the organic layer at the low altitude heath except for the nematodes and the entire organic horizon of about 2 cm at the high altitude heath. SOM (soil organic matter); PLFA (phospholipid fatty acids); f/b (fungal to bacterial ratio); ND (no determination)

		Low altitude heath	High altitude heath
Microbial C	mg C g^{-1} SOM	5.9	11.0
	g C m^{-2}	40.8	28.5
PLFA	mg C g^{-1} SOM	5.3	8.6
	g C m^{-2}	36.2	22.4
Protozoa	mg C g^{-1} SOM	0.11	ND
	g C m^{-2}	0.8	ND
Nematodes			
0–3 (0–2) cm	mg C g^{-1} SOM	0.020	0.017
	g C m^{-2}	0.056	0.039
3–6 cm	mg C g^{-1} SOM	0.013	–
	g C m^{-2}	0.047	–
f/b of PLFA	0–10 (0–2) cm	0.19	0.19
f/b of nematodes	0–3 (0–2) cm	0.20	0.15
	3–6 cm	0.31	–

Table 3
Microbial biomass (Mic) C_{PLFA}, protozoa and nematode biomass in percentage of microbial biomass (Mic) C_{CFE}, the fungal to bacterial biomass ratio and the ratio of fungal to bacterial feeding nematodes (f/b) and the number of protozoa and nematodes per m^2 in the upper 10 (0–3, 3–6) cm of the organic layer of a low altitude heath ($n = 6$, PLFA = 5). ND (no determination)

	$MicC_{CFE}$ (gC m^{-2}) (depth, cm)	$MicC_{PLFA}$ (% of $MicC_{CFE}$) (depth, cm)	f/b (depth, cm)	Protozoa (Ind $\times 10^9$) (depth, cm)	% MicC (depth, cm)	Nematode (Ind $\times 10^6$) (depth, cm)	% MicC (depth, cm)	f/b (depth, cm)	Nematode (Ind $\times 10^6$) (depth, cm)	% MicC (depth, cm)	f/b (depth, cm)
	0–10	0–10	0–10	0–10	0–10	0–3	0–3	0–3	3–6	3–6	3–6
Control (0)	40.8	89	0.19	31.3	1.86	2.3	0.34	0.20	2.1	0.22	0.32
Carbon (C)	53.8	67	0.21	20.2	0.90	3.3	0.30	0.23	2.2	0.23	0.23
Fertilizer (F)	42.4	84	0.17	14.1	0.81	4.0	0.59	0.33	3.6	0.50	0.25
C × F	53.8	78	0.20	23.7	1.03	4.6	0.39	0.28	2.7	0.23	0.15
Benomyl (B)	38.6	73	0.23	14.0	0.89	2.2	0.30	0.31	1.4	0.13	0.37
C × B	52.4	75	0.21	26.8	1.19	1.5	0.13	0.28	2.9	0.21	0.27
F × B	42.5	80	0.18	24.1	1.30	1.7	0.26	0.16	1.8	0.18	0.14
C × F × B	48.6	88	0.19	ND	ND	1.2	0.09	0.73	1.8	0.14	0.24
Bactericide (SP)	39.9	71	0.20	ND	ND	2.6	0.38	0.33	1.6	0.12	0.52
B × SP	44.2	83	0.20	ND	ND	0.8	0.16	0.18	1.7	0.19	0.35

The effects on the microbial community were more evident at the high altitude site (Fig. 2b), where the first principal component both had a significant carbon × fertilizer interaction ($P < 0.01$) as well as a fungicide effect ($P < 0.05$). Carbon and especially carbon plus fertilizer addition shifted the treatments to the left of the PC graph, while the addition of the fungicide shifted the treatments to the right. The second component differed between fertilized and non-fertilized treatments ($P < 0.05$), with the former treatments found in the lower part of the plot. The first principal component accounted for 26.7% and the second for 16.1% of the total variation in the PLFA data.

The treatments, where bacterial antibiotics were applied singly or in combination with benomyl, were not included in the statistical analysis. However, as expected in both areas these treatments clustered together with other treatments without carbon and fertilizer addition.

Fig. 2c shows the loading values for the individual PLFAs at the low altitude heath site. The two PLFAs indicating eucaryotic organisms (18:2ω6,9 and 18:1ω9) decreased with application of fertilizer and increased with carbon addition. The fertilizer treatments increased the relative abundance of the PLFAs i15:0 and a15:0, while carbon addition especially decreased i17:0, a17:0, br18:0 and 10Me16:0.

A similar loading plot for the high altitude site (Fig. 2d) also indicated that 18:2ω6,9 and 18:1ω9 covaried, because they are placed near each other in the plot. Both increased in the carbon-amended plots, also in combination with fertilizer addition, and decreased in the fungicide treatments. The fertilized plots were further characterized by high relative amounts of a number of branched PLFAs, indicative for gram-positive bacteria (e.g. 10Me16:0, 10Me17:0, 10Me18:0, i16:0, i16:1, i17:0 and br18:0), while several PLFAs common in gram-negative bacteria appeared to decrease (e.g. 16:1ω7c, 18:1ω7, 16:1ω5 and cy17:0).

3.3. Protozoa

The numbers of protozoa in the low altitude heath soil ranged between 2.0 and 4.5×10^6 g^{-1} SOM or between 13.8×10^9 and 31×10^9 per m^2 to a depth of 10 cm (Table 3). No treatment differences were found due to very high variation in the material. The protozoan biomass was between 0.05 and 0.1 mg g^{-1} SOM, which is approximately 1.9% of the microbial biomass (Table 3).

3.4. Nematodes

Nematode population density at the control plots of the low altitude heath site showed an average of 680 and 470 individuals g^{-1} DW at 0–3 (Fig. 3a) and 3–6

cm (data not presented) soil depth, respectively. This corresponds to 2.3 and 2.1 × 10^6 ind m^{-2} at 0–3 and 3–6 cm depth (Table 3). At the high altitude heath, population density estimated to a depth of 2 cm was 260 ind g^{-1} DW (Fig. 3b) or 1.5 × 10^6 ind m^{-2} (Table 4). At both sites, addition of fertilizer alone and in combination with carbon led to higher population densities (Fig. 3a and b, Tables 3 and 4). At the low altitude heath, nematode density was twice as high in the 0–3 cm horizon following both these treatments (Fig. 3a). In the 3–6 cm horizon, fertilization resulted in a doubling of the numbers, too, whereas the effect of combined fertilizer plus carbon treatment was lower with an increase of one-third (Table 3). At the high altitude site there was a significant main effect of fertilization ($P < 0.05$), with an increase of about 25% after fertilization alone, but a doubling when fertilizer was combined with carbon addition (Fig. 3b).

A strong decline of the population density took place after application of benomyl. Nematode numbers were reduced 20–60% in the upper soil horizon of the low altitude heath by all treatment combinations containing benomyl ($P < 0.001$, Fig. 3a) and this effect was still prominent at 3–6 cm depth ($P < 0.01$). Nematodes at the high altitude site showed a similar response with numbers reduced by 40–85% in treatments containing benomyl ($P < 0.001$, Fig. 3b). In contrast, the application of the two bactericides did not affect the nematode communities in any of the soils.

The trophic structure of the nematodes was altered by the treatments. Generally, bacterial feeders were the most dominant trophic group with an average of 61% in the control plots at both sites. Fungal feeders were less frequent with 16 and 9% in the low and high altitude heath, respectively. The ratio of fungal to bacterial feeders ranged between 0.15 and 0.32, indicating a bacterial based foodweb in the soils (Tables 3 and 4). At the low altitude heath, both fungal and bacterial feeders increased after application of fertilizer, also in combination with carbon, whereas there was no significant changes in f/b ratio in these treatments (Table 3, Fig. 3c and e). The nematode community at the high altitude site was affected differently. Bacterial feeders did not respond significantly to application of fertilizer or carbon (Fig. 3d). In contrast, fungal feeders showed a strong positive response to fertilization ($P < 0.001$, Fig. 3f) with an increase of about 8-fold in the fertilizer and 13-fold in the fertilizer plus carbon treated soils. This resulted in a strong increase of the fungal to bacterial ratio (Table 4).

Benomyl decreased the bacterial more than fungal feeding nematodes at both sites (Fig. 3c and d), most strongly after combined benomyl and fertilizer application with or without carbon. A moderate decrease in fungal feeders occurred at the low altitude heath after all benomyl-containing treatments in 0–3 cm (Fig. 3e, $P < 0.05$). At the high altitude site, the addition of benomyl alone resulted in a significant reduction of fungivore nematodes. In contrast, they slightly increased when benomyl was added together with fertilizer and carbon (Fig. 3f).

The estimated nematode biomass C was highest at the low altitude heath, where it constituted 0.34 and 0.22% of the biomass C measured for micro-organisms in 0–3 and 3–6 cm, respectively (Table 3). At the high altitude site, the proportion of nematode biomass to that of the micro-organisms was 0.16% (Table 4). Fertilizer increased the proportion of nematode biomass to that of the micro-organisms at the low altitude heath, whereas carbon decreased the proportion at the high altitude heath.

Table 4
Microbial biomass (Mic) C_{PLFA} and nematode biomass C in percentage of microbial biomass (Mic) C_{CFE}, the fungal to bacterial biomass ratio and the ratio of fungal to bacterial feeding nematodes and the number of nematodes per m^2 in the organic layer of a high altitude heath ($n = 6$, PLFA = 4)

	$MicC_{CFE}$ (gC m^{-2}) (depth, cm)	$MicC_{PLFA}$ (% of $MicC_{CFE}$) (depth, cm)	f/b (depth, cm)	Nematode (Ind × 10^6) (depth, cm)	Nematode (% MicC) (depth, cm)	f/b (depth, cm)
	0–2	0–2	0–2	0–2	0–2	0–2
Control (0)	28.6	78	0.19	1.5	0.16	0.15
Carbon (C)	35.1	64	0.18	0.6	0.05	0.25
Fertilizer (F)	30.5	74	0.17	1.6	0.11	1.40
C × F	51.3	59	0.28	2.7	0.12	1.70
Benomyl (B)	31.3	68	0.15	0.3	0.04	0.27
C × B	30.9	82	0.16	0.4	0.04	0.55
F × B	29.8	67	0.14	0.2	0.03	2.40
C × F × B	45.5	67	0.17	0.4	0.02	5.50
Bactericide (SP)	32.8	69	0.17	1.4	0.14	0.26
B × SP	26.5	69	0.17	0.7	0.12	0.39

4. Discussion

4.1. Responses in micro-organisms to increased nutrient and carbon availability

As expected, we found the most pronounced effects of the treatments at the high altitude heath where the amount of added substances per unit SOM was almost three times higher than at the low altitude site. Moreover, the dense moss cover may have absorbed part of the additions at the lower site. The main purpose of monthly addition of carbon and nutrients was to increase the level of available carbon and/or nutrients in the soil permanently during the growing season to minimize resource limitations of the decomposers and the soil fauna. Wu et al. (1993) found that adding plant residues (ryegrass) instead of glucose to a soil resulted in a more persistent biomass increase over a 100 day incubation period, without any displacement of the native biomass. Still, we chose to add a simple carbon source (sucrose) for mainly two reasons. First, in this way we achieved a clear separation between the carbon and nitrogen and phosphorus source, since all plant residues consist of at least small amounts of N

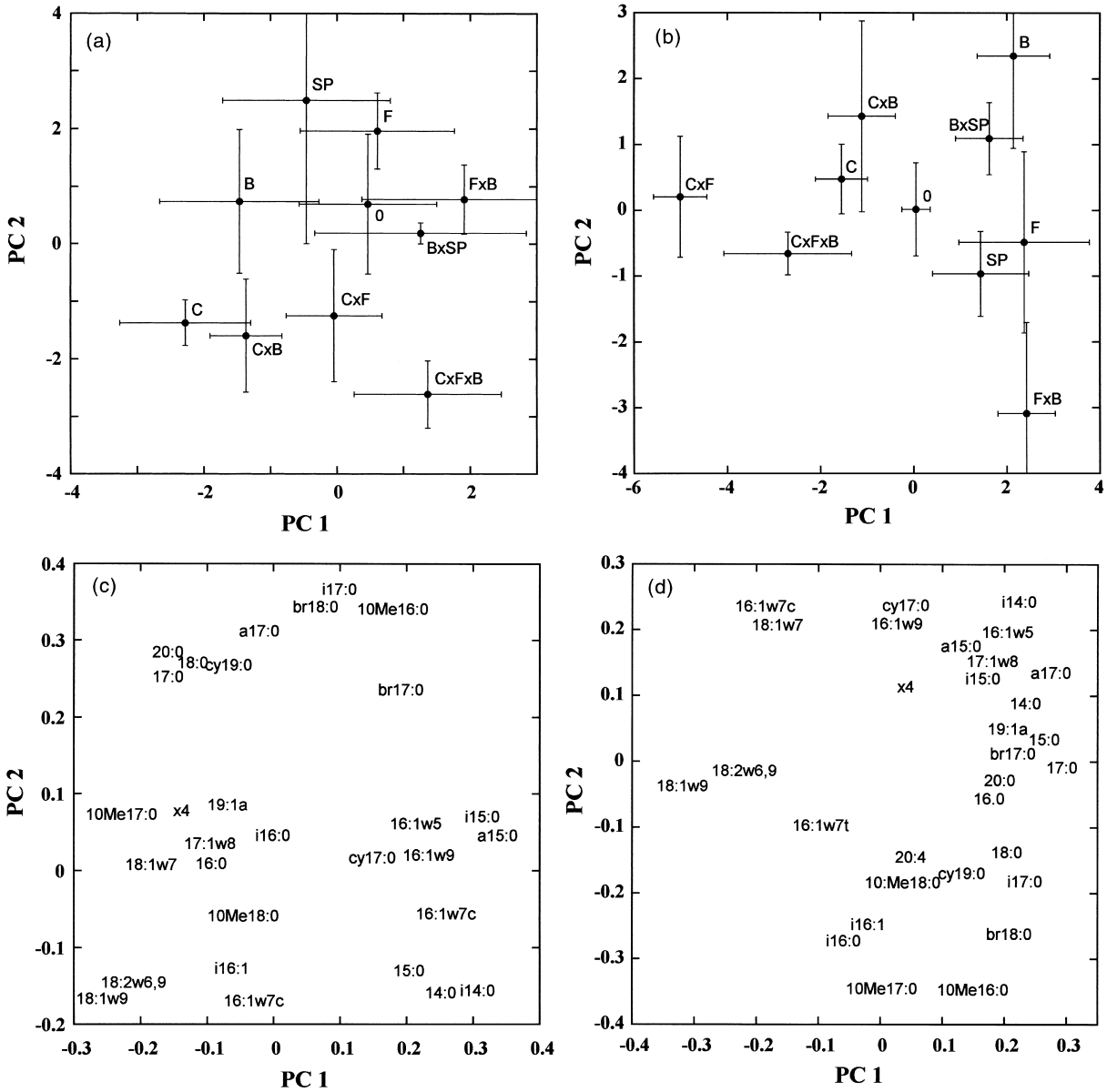

Fig. 2. Principal component analysis of mol% of PLFAs showing score plots for the different treatments (a, b) and loading values for the individual PLFAs (c, d) at a low (a, c) and a high altitude heath (b, d). Abbreviations for (a) and (b), see Table 3. Unidentified PLFAs in (c) and (d) are designed ×4, 19:1a (position of double bond not determined) and br17:0 and br18:0 (unknown methyl branching position). Bars indicate standard errors (n = 5 and 4 for low and high altitude heath, respectively).

and P. Second, sucrose was added every month during the growing season, which was thought to enable the biomass to increase fast and persist during the growing season.

The access to extra carbon increased the CO_2 efflux (Illeris and Jonasson, 1999) and as shown here, the standing crop of micro-organisms gave similar responses with the CFE- or PLFA-technique for microbial biomass C estimates. The effect of carbon addition (Fig. 1) at the low altitude heath indicates that the microbes were primarily limited by carbon. In contrast, it appears that the microbes were closer to nutrient limitation at the high altitude site. This is indicated by the small effect of carbon addition on microbial biomass C (Fig. 1d and f), but a strong increase in DOC (Fig. 1b), presumably mirroring that a large part of the carbon added was still present in the soil. However, because the microbial C increased strongly when nutrients were added together with carbon, coinciding with a decline of DOC to the level in

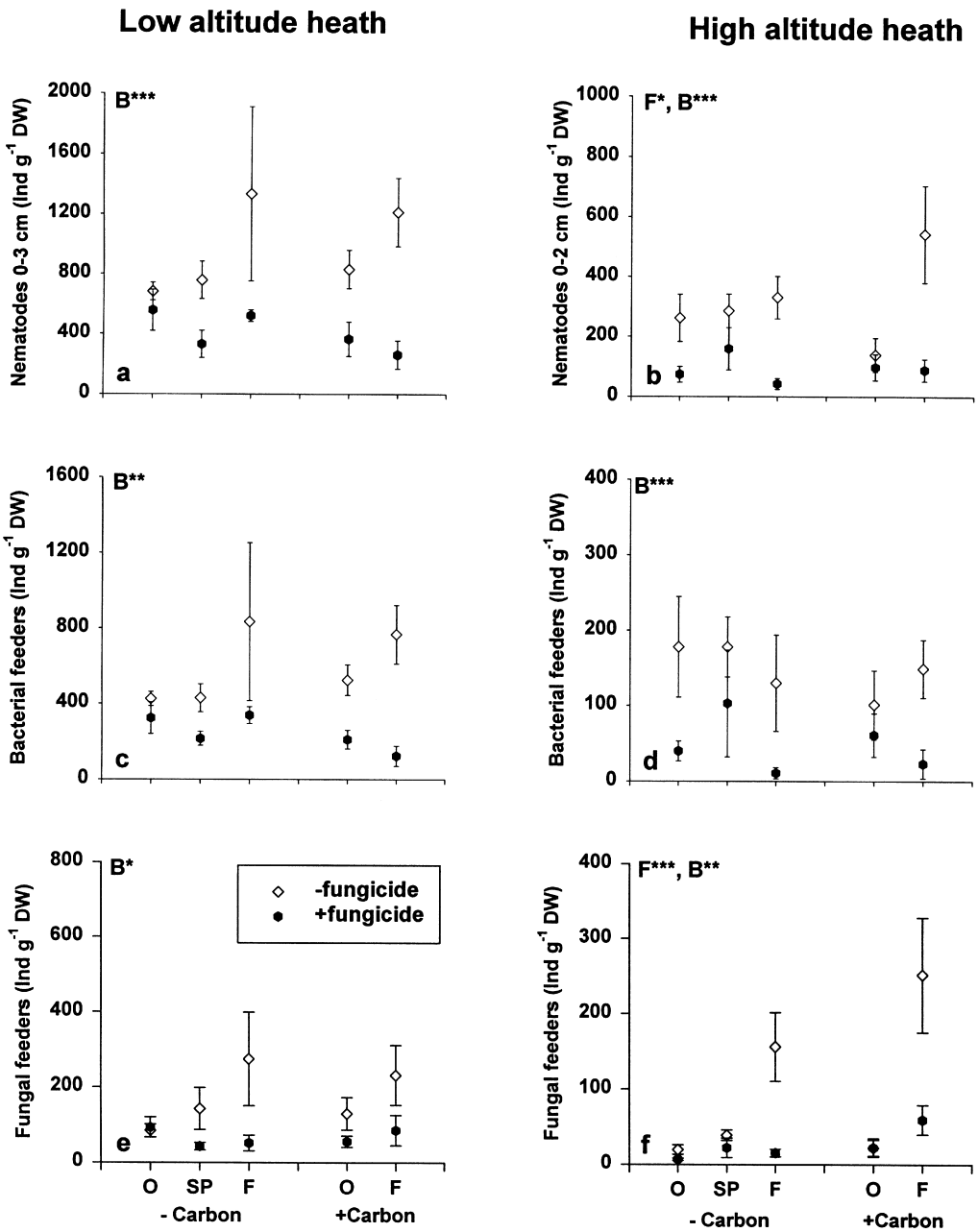

Fig. 3. Nematode density (a, b) partitioned into bacterial (c, d) and fungal feeders (e, f) in August 1996 after four growing seasons with application of benomyl (B), carbon (C), bactericides (SP) and fertilizer (F) and in control plots (0) at a low and a high altitude heath (means ± S.E., $n = 6$). Results of ANOVAs in the factorial design with significant effects of main factors benomyl (B), carbon (C) and fertilizer (F) and interactions are given at the top of the diagram. Levels of significance: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

plots without carbon addition, it appears that the carbon was used only when the nutrient availability was also high. The difference in responses between the heaths could be because the soil organic matter at the high altitude site contained a much higher fraction of labile, non-recalcitrant C than the low altitude soil (Schmidt et al., 1999). Hence, the microbes had access already to a relatively easily degradable C source. The manipulations resulted in similar tendencies in both assessments of microbial biomass, but the PLFA measurement resulted in approximately 70–80% of the amount of C estimated from the CFE-method. The discrepancy between the two approaches probably is due to a non-exact extractability factor for the microbial C estimated by the CFE-technique and/or a non-exact conversion factor when nmol PLFA was converted to microbial biomass C. Both conversion factors are from studies in non-arctic ecosystem. However, the biomass conversions for the protozoa and nematodes are even more uncertain and the conversion factors, for example, for the nematodes differ with an order of magnitude from 0.125 to 0.013 μg C ind^{-1} (Freckman and Mankau, 1986; Maxwell and Coleman, 1995; Sohlenius et al., 1997). Due to the weaknesses in absolute biomass measurements the discussion of grazer pressure will therefore concentrate on relative effects of the treatments.

4.2. PLFA distribution

Although the microbial biomass was unaffected by benomyl or fertilizer addition when not combined with the addition of extra carbon, there were significant effects on the microbial community structure as judged from an altered PLFA pattern (Fig. 2a and b). That changes in the community composition can take place without a subsequent change in total biomass has earlier been found using PLFA measurements, e.g. after metal addition (Bååth et al., 1998).

Changes in individual PLFAs indicated that to some degree the different treatments induced similar changes in the microbial communities at the two sites. The PLFAs 18:2ω6,9 and 18:1ω9, indicative of fungi, had high loadings in carbon treated plots, indicating that these PLFAs increased in both areas when the total biomass increased. In the combined carbon and fertilizer treatment at the high altitude heath, the fungal component accounted for more than 50% of the increase in microbial biomass. However, if benomyl was added too, there was no change in the fungal to bacterial biomass ratio, reflecting that groups of the fungal component were inhibited. The use of 18:2ω6,9 as a signal for fungi has been criticized, as it is associated with many eucaryots and it is abundant in plant tissue as well (Zelles, 1997). However, our results suggest that the observed responses are reliable as a signal of fungal changes for several reasons: Firstly, roots were easily removed from the soil at the high altitude site where we saw the strongest responses. Secondly, the observed responses in 18:2ω6,9 did not reflect plant responses (Michelsen et al., 1999) and, thirdly, combined carbon and fertilizer addition is not likely to make major increases in root biomass compared to the combination with benomyl. The limitation in the use of 18:2ω6,9 is probably more that it is a quantitative measurement and cannot be related to qualitative changes within the fungal community, so we cannot evaluate if the changes are associated with, for example, yeast or mycorrhizal fungi.

Fertilizer increased a group of PLFAs associated with gram-positive bacteria in the high altitude heath (10Me16:0, 10Me17:0, 10Me18:0, i16:1, i16:0, i17:0, br18:0), which all clustered together at low values along PC2 (Fig. 2d). Several of these PLFAs also clustered together in the top right corner in the PC analysis of the low altitude heath (Fig. 2c), indicating that they were more common in fertilizer treatments, especially without carbon addition. PLFAs indicative of gram-positive bacteria have earlier been associated with low pH due to acidification (Pennanen et al., 1998). Increases in fungal to bacterial feeding nematodes, which we observed in the fertilized plots especially at the high altitude heath (Table 4 and Fig. 3d and f), have also been associated with decreased soil pH (Ruess et al., 1996). However, we did not find any changes in the bulk soil pH after fertilizer addition at any of the heaths (data not shown). Interestingly, this group of PLFAs clustered together in a study of the spatial structure of PLFAs in a birch–spruce forest and also aggregated on a smaller scale than other PLFAs, indicating that they formed a complex of associated micro-organisms reacting similarly to environmental conditions (Saetre and Bååth, 2000).

Earlier results on effects of nitrogen fertilization in coniferous forest soils have indicated reduced microbial biomass in acid humus soils up to 10 years after application of 150–600 kg N ha^{-1} (Bååth et al., 1981; Söderström et al., 1983; Nohrstedt et al., 1989; Arnebrant et al., 1996). This was not the case on either of the Abisko heaths (Fig. 1), even if the total nitrogen addition during the four years before sampling added up to 40 g m^{-1} (equivalent to 400 kg N ha^{-1}). A possible explanation for this difference is that in the former studies only nitrogen was added, while the Abisko studies used N:P:K, but results from application of N:K alone to the heath soils showed no changes in microbial biomass C (unpublished data) which suggests that there are fundamental differences in the reaction to nitrogen by the microbial communities in these two soil types, arctic heath and coniferous forest humus.

4.3. Microbivore grazing and nutrient turn-over

The increase in microbial biomass at the low altitude heath in the carbon alone treatment was reflected in nutrient availability, which decreased (Michelsen et al., 1999). The increase was largest in eucaryotic PLFAs and is most likely growth of sugar fungi, including yeast, which have low contributions to decomposition and nutrient mineralization. Furthermore, the increase in micro-organisms was not followed by a similar increase in the microbivores, which in turn probably resulted in slightly lower grazer pressure (Table 3). This was in accordance with measurements of plant growth parameters, which also indicated lower nutrient turn-over after carbon addition at the low altitude heath.

The strong increase of nematode density after fertilizer addition both with and without carbon addition is similar to earlier reported effects of NPK applications in short- and long-term studies in other ecosystem types (Sohlenius and Boström, 1986; Dmowska and Ilieva, 1995; Wasilewska, 1995; Gilbert et al., 1998). In addition, we know that CO_2 evolution increased and stayed high for at least two weeks after addition (Christensen et al., 1998) and plant biomass production was enhanced after fertilizer addition to our sites (Michelsen et al., 1999). It is likely that the large increase in microbivores reflected an increase in microbial biomass production and/or a higher quality of the food source as the nutrient content in the microbial biomass increased after fertilizer application (Michelsen et al., 1999).

4.4. Selective inhibition of the fungal and bacterial biomass and the relationship to nematode feeding groups

We were unable to detect any effect of bacterial antibiotic addition on microbial biomass C, the f/b biomass ratio of microbes, PLFA pattern or on bacterial feeding nematodes. This can either be because of high adsorption of the antibiotics in these organogenic soils, combined with short half-life, or because only a minor fraction of the target group was sensitive to the inhibitors (Hu and van Bruggen, 1997). Of these possible explanations, the lack of changes in the PLFA pattern suggests that the amount added was not sufficient. Ingham et al. (1986) could not observe any significant reduction of bacterial or nematode numbers in a field experiment with addition of about twice the amount we used and Alphei et al. (1995) found that considerably higher concentrations were necessary in carbon-rich soils. Also, streptomycin and penicillin do not kill existing bacteria but inhibit the synthesis of new cells so that the action depends on the microbial growth rate and is detectable mainly when the soil microbial biomass increases rapidly (Wardle and Parkinson, 1990).

In contrast, benomyl is known to be very persistent in soils and, hence, has a longer half-life. In spite of the lack of changes in the microbial biomass C after benomyl addition, the large and unexpected decrease in nematode numbers in both investigated soils indicated that the fungicide indeed affected the soil organisms (Fig. 3, Table 4). Reduced grazing by microbivores has been associated with decreased nutrient turn-over probably due to an older microbial biomass (Clarholm, 1985; Freckman 1988), which is in accordance with the decrease in inorganic nutrients observed in the benomyl treated plots (Michelsen et al., 1999). In contrast, the lack of reduction in vegetation cover or plant tissue nutrient concentration does not suggest lower N and P turn-over rates in the presence of benomyl. Also field application during 19 years of benomyl had no measurable effect on soil microbial biomass or on C or N mineralization (Hart and Brookes, 1996).

Benomyl reduced the population density of all feeding groups of nematodes, including bacterial, fungal and plant feeding species and it appeared from the increasing f/b ratio that the decrease was larger in the bacterial feeding nematodes than in the fungal feeders. Hence, it appears that benomyl, beside affecting fungi, also affects other soil organisms negatively (Jamieson and Killham, 1994). This effect, obviously, has no relation to any reduction of their fungal food source as the loading pattern of the two eucaryotic PLFAs 18:2ω6,9 and 18:1ω9 showed (Fig. 3c and d). Fungal feeding nematodes were dominated by the genus *Aphelenchoides*, which made up 72 and 96% of this trophic group at the low and high altitude site, respectively, and benomyl treated plots showed highest dominance of these nematodes. *Aphelenchoides* ssp. is reported to be toxitolerant against heavy metals and characteristic for heavily polluted roadsides (Sturhan et al., 1986; Weiss and Larink, 1991; Steiner, 1994) and may also be less sensitive to benomyl.

4.5. Conclusion

The elevated levels of DOC and inorganic nutrients (Michelsen et al., 1999) in the soil indicated that the additions of carbon and nutrients increased the levels of these resources at least until the sampling one month later. The response in microbial biomass at the low altitude site after carbon, but not after fertilizer addition, indicates that microbes were C and not nutrient limited. However, the large reduction of DOC coincident with increased microbial C after the combined carbon and NPK addition at the high altitude site indicate that the microbes in this soil were close to nutrient limitation. They could respond strongly to the carbon addition only when nutrients were also added.

Nematode numbers increased strongly after fertili-

zer, but not after carbon application indicating that the quality of the food, e.g. the nutrient content in the micro-organisms, affected nematode density more than the quantity of the food source.

The use of selective inhibitors did not give the effects we had expected. We were not able to detect any effects of the bactericides. Benomyl exerted its major effect on non-target groups, e.g. the nematodes with the lowest effect on fungal feeding nematodes, most likely due to increased abundance of the insensitive *Aphelenchoides* ssp.

Although measurement of grazing pressure can only be inferred from density changes in the nematodes and protozoa, the observed correlation between nutrient availability and nematode grazing pressure suggests that nematodes have an important impact on the turnover rates.

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