Oecologia (1996) 106:507–515

Sven Jonasson · Anders Michelsen · Inger K. Schmidt
Esben V. Nielsen · Terry V. Callaghan

Microbial biomass C, N and P in two arctic soils and responses to addition of NPK fertilizer and sugar: implications for plant nutrient uptake

Received: 12 March 1995 / Accepted: 11 November 1995

Abstract The soil microbial carbon (C), nitrogen (N) and phosphorus (P) pools were quantified in the organic horizon of soils from an arctic/alpine low-altitude heath and a high-altitude fellfield by the fumigation-extraction method before and after factorial addition of sugar, NPK fertilizer and benomyl, a fungicide. In unamended soil, microbial C, N and P made up 3.3–3.6%, 6.1–7.3% and 34.7% of the total soil C, N and P content, respectively. The inorganic extractable N pool was below 0.1% and the inorganic extractable P content slightly less than 1% of the total soil pool sizes. Benomyl addition in spring and summer did not affect microbial C or nutrient content analysed in the autumn. Sugar amendments increased microbial C by 15 and 37% in the two soils, respectively, but did not affect the microbial nutrient content, whereas inorganic N and P either declined significantly or tended to decline. The increased microbial C indicates that the microbial biomass also increased but without a proportional enhancement of N and P uptake. NPK addition did not affect the amount of microbial C but almost doubled the microbial N pool and more than doubled the P pool. A separate study has shown that CO_2 evolution increased by more than 50% after sugar amendment and by about 30% after NPK and NK additions to one of the soils. Hence, the microbial biomass did not increase in response to NPK addition, but the microbes immobilized large amounts of the added nutrients and, judging by the increased CO_2 evolution, their activity increased. We conclude: (1) that microbial biomass production in these soils is stimulated by labile carbon and that the microbial activity is stimulated by both labile C and by nutrients (N); (2) that the microbial biomass is a strong sink for nutrients and that the microbial community probably can withdraw substantial amounts of nutrients from the inorganic, plant-available pool, at least periodically; (3) that temporary declines in microbial populations are likely to release a flush of inorganic nutrients to the soil, particularly P of which the microbial biomass contained more than one third of the total soil pool; and (4) that the mobilization-immobilization cycles of nutrients coupled to the population dynamics of soil organisms can be a significant regulating factor for the nutrient supply to the primary producers, which are usually strongly nutrient-limited in arctic ecosystems.

Key words Arctic/alpine soils · Benomyl · Microbial C, N, P · Nutrient immobilization · Plant nutrient uptake

S. Jonasson (✉) · A. Michelsen · I.K. Schmidt · E. Nielsen
Botanical Institute, Department of Plant Ecology,
University of Copenhagen, Øster Farimagsgade 2D,
DK–1353 Copenhagen K, Denmark

A. Michelsen
Institute of Terrestrial Ecology, Merlewood Research Station,
Grange-over-Sands, Cumbria LA11 6JU, UK

T.V. Callaghan
Sheffield Centre for Arctic Ecology,
Department of Animal and Plant Sciences,
The University of Sheffield, Tapton Experimental Gardens,
26 Taptonville Road, Sheffield S10 5BR, UK

Introduction

A large number of reports have shown that addition of fertilizer generally induces a strong increase in the productivity of arctic plant communities (e.g. Warren Wilson 1966; Haag 1974; Shaver and Chapin 1980, 1986; Jonasson 1992). The response has been taken as an indication that plant growth in the Arctic is strongly nutrient-limited (Ulrich and Gersper 1978; Shaver and Chapin 1980, 1986). It has, however, also been observed that the fertilizer must be added in relatively large amounts to stimulate the plant productivity. For instance, addition of 5 g m^{-2} of nitrogen (N) and phosphorus (P), equalling approximately 3–5 times the annual incorporation of N into the vegetation and 20–40 times the annual uptake of P, gave small responses in plant growth, and low nutrient recovery in the vegetation, but high recovery in the soil (Marion et al. 1982; Shaver et al. 1986; Jonasson and Chapin 1991). After addition of considerably larger amounts of up to 25 g m^{-2} N and P, Shaver and Chapin (1980) observed, however, a substantial fertilizer effect

on the vegetation. It has been proposed that the limited plant growth response to additions of moderate amounts of nutrients to arctic soils is because nutrients could have been immobilized by the soil microorganisms (Marion et al. 1982; Jonasson and Chapin 1991), but to our knowledge this has not been properly tested. The implications of this probable high nutrient immobilization by soil microorganisms are important for a correct understanding of the nutrient circulation in tundra for two main reasons. Firstly, microorganisms in arctic soils may not only supply nutrient to the plants through mineralization of soil organic matter, but may also reduce the amounts of inorganic, plant-available nutrients during periods of high microbial demand. Secondly, the microbes could act as an important "buffer" against soil eutrophication (Jonasson in press). This applies for instance to the eutrophication which, as has been proposed, may result from the increased soil temperature and nutrient mineralization induced by changes in global climate (Post 1993).

Inferences about nutrient cycling in the Arctic are most often based on measurements of nutrients in the vegetation and litter, in the inorganic, plant-available soil pool, and/or in the bulk soil, and on estimates of fluxes of nutrients between these pools. Little is known, however, about the nutrient content of the soil microorganisms, which are the regulators of the nutrient fluxes between the biologically important soil pools, and even less is known about controls on microbial biomass production, nutrient uptake and nutrient release. Both estimates of microbial carbon (C) and nutrient pools (Smith and Paul 1990) and suggestions about the detailed role of the microorganisms in the nutrient immobilization-mobilization cycle have been inferred from indirect measurements (e.g. Marion et al. 1982; Shaver et al. 1986; Jonasson and Chapin 1991; Jonasson et al. 1993; Shaver and Chapin 1995). For instance, as data are lacking, even recent reviews of microbial biomass and microbial nutrient content (Smith and Paul 1990) use the sparse data for the arctic regions that were already available some 20 years ago (Rosswall 1976). These data are calculated from microbial densities and assumed average nutrient contents across the broad groups of soil microorganisms rather than based on direct estimates, for which there were no reliable methods at that time.

New techniques, e.g. the fumigation-extraction method, for direct measurements of microbial biomass C and nutrients are now available and have been thoroughly tested during the last 10–15 years (e.g. Jenkinson and Powlson 1976; Brookes et al. 1982, 1985a, b; Tate et al. 1988), but we are aware of only one study (Cheng and Virginia 1993) in which this technique has been used for estimations of microbial C and nutrients (N) in arctic ecosystems.

In this study we use the fumigation-extraction method to quantify the amount of microbial C in two contrasting arctic soils and to estimate the proportions of N and P in the soil microbial and inorganic pools. Secondly, we examine how the C, N and P pools are affected by factorial addition of NPK fertilizer and labile carbon (sugar). By these additions we want to determine the sink-source potential for nutrients in the soils and investigate how the microbial sink strength for nutrients through microbial immobilization-mobilization processes is controlled by the availability of labile carbon and easily assimilated nutrients. We also included addition of a fungicide, benomyl, which has previously been shown to affect arbuscular mycorrhizal and pathogenic fungi (Fitter 1986; Koide et al. 1988), to examine whether this compound could be used for manipulating the soil inorganic and the fungal nutrient pool sizes in arctic soils. (We also wanted to examine whether the addition of benomyl would reduce the benefit to the plants of mycorrhizal colonization. This, however, is beyond the scope of the present paper.)

We do not include data on growth or nutritional responses in the plants because the dominant species in these arctic communities respond slowly to nutritional changes (Shaver and Chapin 1980). Analyses of plant nutrient responses are planned after a period of time when nutritional responses are likely to be manifested and will be reported at a later date.

Methods

Site description

The experiment took place from spring to autumn 1993 at two sites near Abisko Scientific Research Station in North Sweden (68°21′N, 18°49′E). One site was a low alpine heath at the tree limit about 450 m above sea level (a.s.l.) with a pH_{H2O} of 7.1 (Table 1) and a 15–20 cm deep layer of organic matter. The heath was dominated by ericaceous dwarf shrubs, most commonly *Vaccinium uliginosum*, *Empetrum hermaphroditum*, *Rhododendron lapponicum*, *Dryas octopetala* and prostrate dwarf birch (*Betula nana*). Forbs and graminoids were scattered, whereas mosses were abundant and covered most of the ground.

The second site was a high-altitude (1150 m a.s.l.) fellfield with a thin, c. 2-cm-deep discontinuous organic horizon with a mean pH of 5.2 (Table 1) and a more open vegetation dominated by *E. hermaphroditum*, *Cassiope tetragona*, *Vaccinium vitis-idaea*, scattered forbs and a discontinuous moss cover.

Perturbations and sampling

We selected a visually homogeneous area at each site in which we marked six rows representing blocks with eight 1.5×1.5 m quadrats surrounded by a protection zone 1 m wide. On 20 June, in connection with thawing of the soil in spring, we sampled five randomly selected circular soil plugs with a diameter and depth of 4 and 10 cm, respectively, within each quadrat on the heath. On 1 July we sampled the fellfield in a similar way, but because the organic layer was thin we took a varying number of plugs from the humus layer until the total length of the plugs was at least 25 cm. Also, we had to limit the sampling to three blocks due to shortage of time. After sampling, we added 250 g sugar m^{-2}, a mixed NPK fertilizer (5:1.25:3.75 g m^{-2}, respectively), and 4 g m^{-2} benomyl (Du Pont, de Nemours, Belgium), containing 2 g m^{-2} active substance, in a full factorial design. Hence, each block contained three plots with addition of either sugar, NPK fertilizer or benomyl, another three plots with all possible pairwise combinations of these substances, one plot in which all three substances were added and one control with no addition, i.e. 8 plots altogether. The plot positions for each treatment were selected randomly within blocks. The additions were repeated on 30 July (heath) and 3 Au-

Table 1 Soil pH, loss on ignition, N and P content (means±SE) C/N, C/P and N/P ratios in unperturbed plots in spring. Extractability factors of 0.40 for microbial N and P and 0.35 for microbial C were assumed (*SOM* soil organic matter)

Parameter	Heath	Fellfield
pH_{H2O}	7.1±0.04	5.2±0.02
Loss on ignition (%)	81.4±1.2	41.3±2.1
N_{tot} (mg g^{-1} SOM)	22.8±0.4	19.3±0.4
(g m^{-2})	159±3	51±1
N_{inorg} (mg g^{-1} SOM)	0.02±0.003	0.005±0.0003
(g m^{-2})	0.16±0.02	0.01±0.001
N_{mic} (mg g^{-1} SOM)	1.40±0.05	1.40±0.07
(g m^{-2})	9.54±0.43	3.59±0.21
P_{tot} (mg g^{-1} SOM)	1.18±0.02	2.51±0.08
(g m^{-2})	8.2±0.16	6.6±0.20
P_{inorg} (mg g^{-1} SOM)	0.009±0.0004	0.001±0.0001
(g m^{-2})	0.06±0.003	0.004±0.0002
P_{mic} (mg g^{-1} SOM)	0.41±0.01	0.05±0.004*
(g m^{-2})	2.84±0.13	0.120±0.011*
C_{mic}	13.9±0.33	18.1±0.83
C_{tot}/N_{tot}	21.9	25.9
C_{tot}/P_{tot}	424	199
N_{tot}/P_{tot}	19.4	7.7
C_{mic}/N_{mic}	9.9	12.9
C_{mic}/P_{mic}	33.9	362*
N_{mic}/P_{mic}	3.4	28.6

* The low amount of microbial P and the high C/P and N/P ratios in the microbial biomass indicate that the extractability of microbial P was considerably lower than the assumed k_{EP} of 0.40 in the fellfield soil

gust (fellfield) with the same amount of sugar and benomyl added, but with addition of the double amount of the mixed fertilizer.

On 27 August (fellfield) and 3 September (heath) we resampled all quadrats at each site, with six replicates per treatment both at the heath and the fellfield.

Laboratory methods

The soil samples were brought to the laboratory where the stones, roots and macroscopic, undecomposed dead plant parts were removed, after which we used a 40-g subsample to determine the water content gravimetrically by weighing the fresh and dried (70°C) soil. Part of the dried sample was weighed, ashed for 48 h at 550°C and reweighed to determine the loss on ignition. The soil carbon content was estimated by multiplying the loss on ignition by 0.5. The remaining dried soil was kept for analyses of total N by the indophenol method and P by the molybdenum blue method after digesting 250 mg soil in a sulphuric acid-selenous acid mixture for 1 h (Kedrowski 1983).

Within 48 h after collection, during which the soil was kept refrigerated at 3°C, 15 g fresh soil from each quadrat was put in small mesh baskets and placed in a desiccator together with a beaker containing 30 ml of ethanol-free chloroform. The $CHCl_3$ was brought to boiling by evacuation of the desiccator, and the samples were fumigated by the vapour for 42 h to lyse the microbial cells and release C and nutrients from the soil microbial biomass (Jenkinson and Powlson 1976; Tate et al. 1988). The desiccator was flushed repeatedly after completed fumigation and the soil was removed from the baskets, placed in bottles in a rotary shaker and extracted for one hour in 70 ml 0.5 M K_2SO_4. The suspension was placed in a refrigerator overnight, filtered the following morning and the extracts were frozen until their C, N and P content were chemically analysed.

Starting at the same time as the fumigation, another 15 g of fresh soil from each quadrat was extracted as above, but without fumigation, to recover the soil inorganic nutrients.

Inorganic N and P were analysed in the unfumigated extracts by the same methods as for total N and P above. The remaining amounts of the extracts were centrifuged and 10 ml of the centrifuged extracts were digested for 4 h in a 5 ml sulphuric acid+selenous acid mixture with 2 ml H_2O_2 added, filled up with distilled water to 100 ml, and analysed for N and P. The digestion of the fumigated samples mineralizes the organic fractions of microbial N and P plus other suspended organic, nutrient holding constituents. Hence the extractable microbial N and P content can be calculated by subtracting the N and P content in digested, unfumigated extracts from the content in digested, fumigated extracts (Brookes et al. 1982, 1985a, b; Vance et al. 1987). In our analyses, as in previous studies (e.g. Brookes et al. 1982), the digestion of the extract did not give any significant addition of P over undigested extracts, indicating that the extracted microbial P was converted to inorganic form in connection with its release from the microbial biomass. For this reason we used the P analyses of undigested extracts for estimating microbial P.

The C in the fumigated and unfumigated extracts was analysed using a Shimadzu Total Organic Carbon Analyser, TOC-500, and the microbial C was estimated as the difference of C content between fumigated and unfumigated extracts.

Part of the microbial C, N and P cannot be extracted, probably because it is locked into microbial cell walls. Hence, to obtain an estimate rather than an index of the microbial nutrient content, the recovered amount must be corrected for extractability. Here we assume an extractability factor k_{EC}=0.35 for C and a factor k_{EN}=k_{EP}=0.40 for N and P. The k_{EC} factor of 0.35 was recommended by Sparling et al. (1990), and is similar to that used by Cheng and Virginia (1993) for tundra soil. The factor 0.40 for N and P is in the middle of the range of previously reported values of the extractability for N by K_2SO_4 (e.g. Brookes et al. 1985b; Ross and Tate 1993; Voroney et al. 1993), and similar to the commonly used extractability factor for P (Brookes et al. 1982), although with a different extractant.

Computations and statistical analyses

Treatment effects were examined on soil inorganic N and P and on microbial C, N and P concentrations per unit soil organic matter (SOM) by factorial analysis of variance with fertilizer, sugar, benomyl additions and block as factors. In the calculations of treatment effects at the heath by late summer, the June chemical data were included as a covariable to compensate for initial differences in chemical content among the plots. This was not possible for the testing of treatment effects at the fellfield because the June measurements there were restricted to three of the six blocks.

To obtain an area-based measure of the pools, we multiplied the concentration values with the soil mass per unit area of the sampled soil cores and express the C and nutrient pools in mass per unit area. This measure represents the content in the whole volume of the organic horizon at the fellfield, but it is restricted to the upper 10 cm on the heath, within which we considered that the treatment effects should be most pronounced.

Results

Nutrient and carbon partitioning in the soil

At the spring sampling, the heath and the fellfield contained on an average 22.8±0.38 and 19.3±0.38 mg N g^{-1} SOM, respectively (Table 1). The inorganic fraction constituted 0.1% or less of these pools. The microbial biomass at both sites contained 1.40 mg N g^{-1} soil corresponding to 6.1% of the total soil nitrogen pool at the heath and 7.3% at the fellfield. The microbial C content was lower and reached 2.8% (13.9 mg g^{-1} SOM) and

3.6% (18.1 mg g^{-1} SOM) of the total soil carbon content at the heath and fellfield, respectively, assuming a C content of 50% in the SOM. Hence, the C/N ratio of the microbial biomass was 9.9 in the heath soil and 12.9 in the fellfield soil.

The total P content in the heath soil was 1.2±0.02 mg g^{-1} SOM and 2.5±0.08 mg g^{-1} SOM at the fellfield (Table 1) where mineral soil constituted a much larger proportion of the bulk soil than at the heath. On the heath, both the proportions of P in extractable, inorganic form (0.8% of the total in SOM) and in the microbial biomass (34.7% of the total in SOM, corresponding to C/P and N/P ratios of 33.9 and 3.4, respectively) were much higher than the proportion of inorganic and microbial N. Both microbial N and P were within the expected range in microbial biomass (Patra et al. 1990; Smith and Paul 1990).

On the fellfield, both the inorganic and microbial P content were much lower. The microbial C/P and N/P ratios were 362 and 28.6, respectively. The high ratios at the fellfield show that the low P values in the extracts of this soil were mostly due to low extractability by the extractant we used, rather than to low P concentrations *per se*. This has been confirmed by comparisons of P extracted with NaH_2CO_3 as extractant.

The autumn levels of the soil inorganic and microbial N and P and of microbial C in the untreated control plots were not significantly different from the spring levels (P>0.13; data not shown).

Treatment effects

There were no differences (P>>0.05; one-factor ANOVA) in total and inorganic soil N and P or in microbial C, N and P among the untreated plots in June before the perturbations. Hence, the differences in C, N and P fractions in autumn relate to treatment effects and not to differences in initial nutrient contents.

Sugar amendments increased microbial C significantly, by 15% at the heath and 37% at the fellfield (Fig. 1, Table 2) but did not affect the microbial nutrient content (Figs. 2 and 3; Tables 3 and 4). However, there was a tendency for inorganic N values to decline in most cases when sugar was added (Figs. 2 and 3), although this was significant (P=0.0002) only when sugar was added together with fertilizer at the fellfield (Table 3). NPK addition resulted in an increase (P<0.001) of the inorganic and microbial N (Table 3) and P (Table 4) concentrations, except for soil inorganic P content at the heath (P=0.075). In contrast, nutrient addition did not affect the amount of microbial C. However, ongoing research has demonstrated an increase of CO_2 production of about 30% after addition to the heath of NPK and NK in the same amounts that we used (L. Illeris, personal communication) and an increase of more than 50% after sugar addition. The enhanced CO_2 production started within 24 h after the additions and was sustained for at least 2–3 weeks. The lack of change in microbial C after addition of fertilizer in spite of the response in CO_2 production demonstrates that an increase of microbial activity does not necessarily correlate with changes in microbial biomass C. None of the treatments changed the soil pH values.

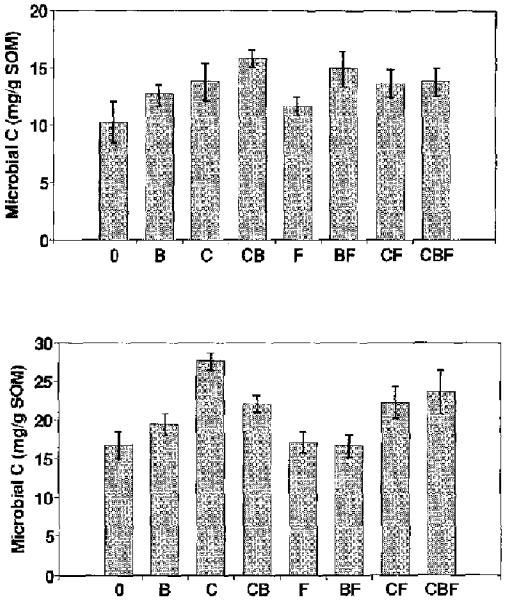

Fig. 1 Late-season microbial carbon content (means±SE) per unit soil organic matter on a low-altitude arctic/alpine heath (*above*) and a high-altitude fellfield (*below*) after one growing season of fungicide (benomyl, *B*), carbohydrate (sugar, *C*) and fertilizer (NPK, *F*) amendment in a factorial design (*0*, control). For treatment effects, see Table 2

Table 2 ANOVA table for treatment effects and interactions on microbial C after one growing season of fertilizer (*F*), carbohydrate (sugar; *C*) and fungicide (benomyl; *B*) amendment to an arctic/alpine heath and fellfield site. Significant effects (P<0.05) are indicated by *italics*

Treatment	Heath		Fellfield	
	F	*P*	*F*	*P*
F	0.57	0.465	0.97	0.761
C	7.22	*0.011*	23.71	*0.000*
B	2.34	0.136	0.48	0.500
F×C	2.02	0.149	0.58	0.460
F×B	0.00	0.983	0.05	0.835
C×B	0.86	0.431	0.15	0.701

Fertilizers and sugar interacted in two cases. On the fellfield a strong increase in inorganic N after addition of NPK fertilizer disappeared when sugar was also added (Fig. 3, Table 3). This indicates that extra nutrients were absorbed by the microorganisms when labile carbon became available and the microbial biomass increased. However, the amount absorbed was too low to produce any significant increase in the relatively large microbial N pool. The same probably occurred with the inorganic P although the interaction was not statistically significant (P=0.109; Table 4) due to a rather large variance of the sample means.

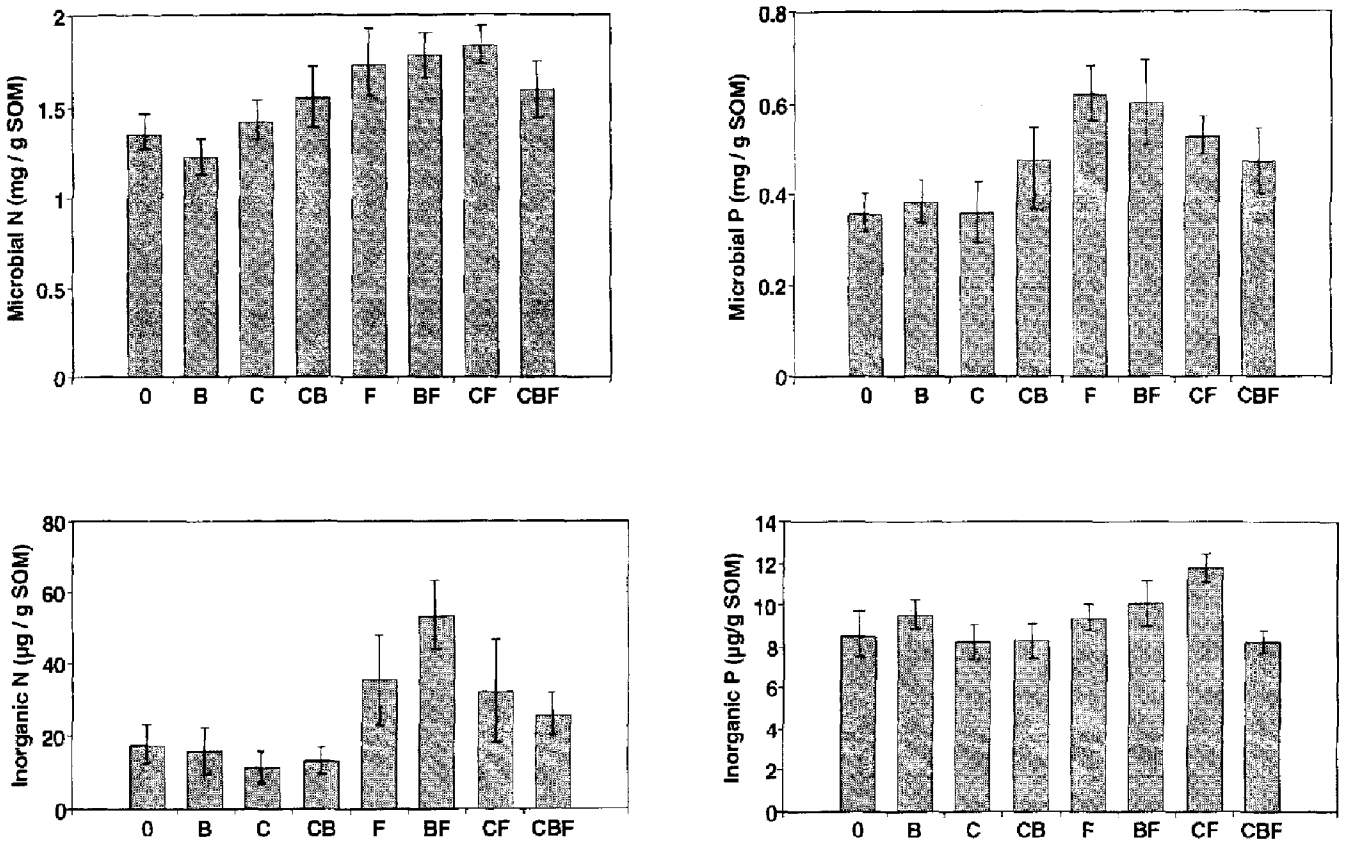

Fig. 2 Late-season inorganic and microbial nutrient concentrations (means±SE) per unit soil organic matter on a low-altitude arctic/alpine heath. *Symbols* and treatments as in Fig. 1; see Tables 3 and 4 for treatment effects

The second interaction between fertilizer and sugar, although weaker, occurred with microbial P on the heath (Fig. 3, Table 4). Fertilization alone caused a strong increase of microbial P, but the effect declined for unknown reasons when fertilizer was applied together with sugar (P=0.024).

Addition of benomyl did not result in any significant changes in microbial C or in inorganic and microbial N and P (Figs. 2 and 3, Tables 2–4), nor were there any significant interaction effects between benomyl, nutrients, or carbohydrates.

Microbial nutrient accumulation and recovery after fertilizer application

The amount of fertilizer N recovered in the soil microbial and inorganic fractions varied between 10.4 and 26.9% on the heath and between 10.7 and 22.5% on the fellfield (Table 5). P was recovered in larger amounts of between 20.0 and 54.1% on the heath, while the recovery could not be determined at the fellfield due to the low extractability. Most of the recovered nutrients were in the microbial biomass.

Discussion

Pool sizes of microbial C, N and P in arctic soils

The microbial N content of 3.6 g m^{-2} that we found at the fellfield (Table 1) corresponds closely to a global av-

Table 3 ANOVA table for treatment effects and interactions on inorganic and microbial N after one growing season of fertilizer (F), carbohydrate (sugar; C) and fungicide (benomyl; B) amendment to an arctic/alpine heath and fellfield site. Significant effects (P<0.05) are indicated by *italics*

Treatment	Heath				Fellfield			
	Inorganic N		Microbial N		Inorganic N		Microbial N	
	F	P	F	P	F	P	F	P
F	*12.50*	*0.002*	*12.31*	*0.002*	*26.61*	*0.000*	*59.72*	*0.000*
C	2.44	0.136	0.55	0.475	*20.06*	*0.000*	0.01	0.918
B	0.22	0.650	0.23	0.645	1.57	0.225	0.14	0.721
F×C	0.78	0.397	1.42	0.247	*18.34*	*0.000*	2.35	0.140
F×B	0.19	0.673	0.21	0.659	0.06	0.809	0.00	0.997
C×B	0.67	0.430	0.01	0.914	0.45	0.517	0.40	0.539

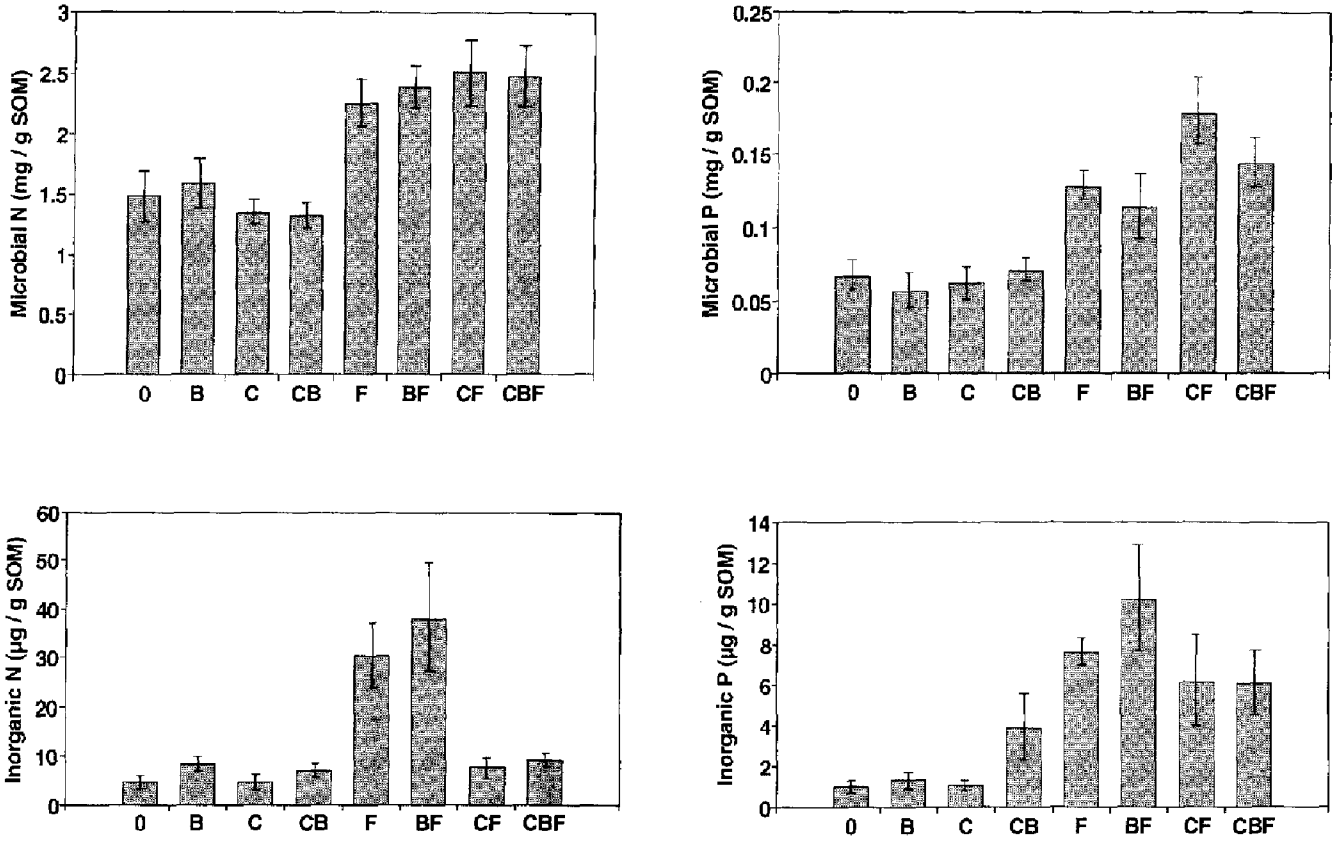

Fig. 3 Inorganic and microbial nutrient concentrations (means ±SE) per unit soil organic matter on a high-altitude arctic/alpine fellfield. *Symbols* and treatments as in Fig. 1; see Tables 3 and 4 for treatment effects

erage of 4 g m^{-2} estimated for tundra soils (Rosswall 1976), whereas the content of 9.5 g N m^{-2} in the upper 10 cm of the heath soil is appreciably above this average. The global arctic average was estimated indirectly by calculations based on assumed averages of microbial densities and microbial N content from observations at a few places in the Arctic. Similarly, assuming 50% C in the microbial biomass, the microbial carbon content of 46 g m^{-2} that we estimated at the fellfield corresponds well with an estimated average of 100 g m^{-2} microbial dry mass across the entire Arctic (Rosswall 1976).

The rather shallow soil at the fellfield is probably more similar to the average for the Arctic, ranging from soils of high organic matter content in the subarctic to soils with low organic matter in the high Arctic, than the heath soil. Hence, the similarity between the average microbial biomass C and N content in arctic soils and the content we found in the fellfield soil indicates that the early, indirect estimates of average microbial biomass and microbial N content in tundra (Rosswall 1976) are realistic. Our estimates are close to microbial C values, ranging from 20.6 to 36.8 mg g^{-1} soil C and C/N ratios of 9.74–13.5 (with an occasional high value of 21.8) reported by Cheng and Virginia (1993) from different soils of a watershed in arctic Alaska. Values of microbial P in arctic soils have not, to our knowledge, been estimated previously.

Table 4 ANOVA table for treatment effects and interactions on inorganic and microbial P after one growing season of fertilizer (*F*), carbohydrate (sugar; *C*) and fungicide (benomyl; *B*) amendment to an arctic/alpine heath and fellfield site. Significant effects ($P<0.05$) are indicated by *italics*

Treatment	Heath				Fellfield			
	Inorganic P		Microbial P		Inorganic P		Microbial P	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
F	3.53	0.075	*25.6*	*0.000*	*21.81*	*0.000*	*33.51*	*0.000*
C	0.14	0.712	0.90	0.365	0.39	0.548	2.71	0.114
B	0.30	0.594	0.32	0.587	1.33	0.262	0.83	0.382
F×C	0.42	0.539	*5.97*	*0.024*	2.80	0.109	1.84	0.189
F×B	1.75	0.201	2.58	0.124	0.01	0.923	0.75	0.404
C×B	3.67	0.070	0.12	0.735	0.00	0.967	0.00	0.973

Table 5 Recovery of fertilizer N and P (g m $^{-2}$) in the inorganic and microbial soil fractions after additions of fertilizer alone (*F*) or in combination with benomyl (*B*) and carbohydrates (*C*) to an arctic/alpine heath and fellfield. The amounts in the microbial fraction assume an extractability of 0.4. Data are not given for P at the fellfield because of poor extractability (see text)

Site	Treatment	N_{inorg}	N_{mic}	Total	% of added	P_{inorg}	P_{mic}	Total	% of added
Heath	F	0.14	3.29	3.43	22.9	0.010	2.02	2.03	54.1
Heath	BF	0.26	3.78	4.04	26.9	0.015	1.91	1.92	51.3
Heath	CF	0.11	4.11	4.22	28.1	0.027	1.39	1.42	37.8
Heath	CBF	0.05	1.51	1.56	10.4	−0.003	0.75	0.72	20.0
Fellfield	F	0.07	1.89	1.96	13.1				
Fellfield	BF	0.07	1.54	1.61	10.7				
Fellfield	CF	0.02	3.35	3.37	22.5				
Fellfield	CBF	0.01	2.33	2.34	15.6				

Nutrient partitioning between soil pools; implications for plant nutrient availability

The inorganic N and P pools – i.e. the nutrient fractions that are instantaneously available for plant and microbial uptake – were small compared to the pools in the soil organic matter and in the microorganisms (Table 1). Also, our analyses showed that a much larger proportion of the total soil P (34.7% at the heath) than of the soil N (6.1–7.3%) and C (3.3–3.6%) was incorporated in the soil microbial biomass, i.e. there is a generally lower N/P and C/P ratio in the soil microbial biomass than in the non-living soil organic matter.

The proportionally high concentration of P in the biomass of soil microorganisms is also reflected in their uptake ratio of fertilizer N and P (Table 5), ranging between 1.63 and 2.95. This is comparable to N/P ratios actually in microbial biomass (Williams and Sparling 1984; Smith and Paul 1990).

Due to the high fraction of P in the microbial biomass in comparison to the total P content of the soil and its relatively labile character (Sarathchandra et al. 1989), any changes in the population size of soil microorganisms should have a much higher effect on the supply of P than that of N to the inorganic, plant-available, nutrient pool. This is consistent with suggestions that microbial processes are particularly important in regulating plant P availability (Chapin et al. 1978; Wood et al. 1984), and implies that short-term availability of P to the plants depends more on changes in population sizes of soil microorganisms than on changes of mineralization rates: episodes of microbial population decline will cause a flush of P to the inorganic pool, whereas phases of population growth will cause strong depletion of the pool. Even though it has been proposed that plant nutrient uptake in arctic soils could be strongly regulated by such pulses (Chapin et al. 1978; Jonasson and Chapin 1991), we have no indications of how common such episodes are. The similar amounts of microbial C, N and P in early spring and autumn demonstrated in this study agrees with results from an ongoing study by us with shorter time intervals between sample collections. Hence, we have not yet found any seasonal pulses of microbial growth or nutrient uptake during the growing season at our sites. Giblin et al. (1991) showed, however, that net mineralization of N and P in several Alaskan soils increased strongly during the winter. Hence, microbial dieback could occur outside the growing season. Whether the inorganic nutrients released during this period are absorbed by the plants in early spring when the soil thaws, are taken up by early season's microbial absorption, or are lost by leaching is yet unknown (see Zak et al. 1990).

Microbial responses after sugar amendments and benomyl addition

The increase of microbial biomass C after addition of labile C indicates that the soil microbes responded by expanding their population size when supplied with extra carbon/energy. The enhanced incorporation of C in the microbial biomass was not followed by any increase of microbial N and P. This was probably because the mobilization rate of these nutrients from the SOM was too low to result in any noticeable extra addition to the proportionally large amounts already incorporated in the microbes when they switched over from the SOM to the added carbon as a main energy source. However, the level of inorganic N in the fertilized soil at the fellfield, where all responses were strongest, declined significantly, and inorganic P tended to decline when labile C was also added (Tables 3 and 4). Furthermore, in several cases the inorganic N and P levels in unfertilized soils also tended to decline after addition of labile C. These trends could not, however, be supported statistically because the concentrations generally were close to the detection limit and the treatment means therefore had a relatively high variance.

In a pot experiment with arctic graminoids grown in soil collected close to the heath site, both soil inorganic N and plant growth declined after addition of leaf extracts of *Empetrum hermaphroditum* (Michelsen et al. 1995). The extracts were rich in labile C, which led to microbial nutrient immobilization and enhanced microbial activity.

The lack of response to the benomyl addition could be due to several reasons. Firstly, the lack of decline in microbial C and nutrient pools could be because benomyl is a fungicide. Hence, it should not affect the bacterial community, which can have a high biomass (Parinkina 1974) and constitute a proportionally large part of the

active microbial biomass in tundra soils (Rosswall et al. 1975). Secondly, benomyl probably acts more strongly on mycorrhizal function, i.e. on N and P absorption, translocation and transfer, than on mycorrhizal fungal colonization of roots and soil (Fitter 1986; Koide et al. 1988). Note that nutrient uptake by plants was not measured in this study, so we have no indication of how strongly the fungicide acted on the mycorrhizal function. Others have also failed to find an effect of the fungicide on soil extractable P (Fitter and Nichols 1988). It is possible that the benomyl is quickly adsorbed to the soil particles and therefore biologically unavailable in soils rich in organic matter (see Liu and Hsiang 1994).

Microbial nutrient immobilization: implications for plant nutrition

In spite of weak responses in microbial biomass C to nutrient addition, the soil microorganisms nevertheless absorbed large amounts of N and P from the fertilizer, which resulted in an approximate doubling of their biomass N and P by 4 weeks after the additions. Furthermore, nutrient addition also stimulated microbial activity, shown by the rapid and persistent increase of CO_2 production after NPK and NK additions (L. Illeris, personal communication). Hence, although the microorganisms responded by increasing their biomass only after addition of labile C, they apparently increased their activity when the pools of inorganic nutrients increased. This is the first *direct* evidence of which we are aware that microbial activity increases in response to fertilizer addition to arctic soils, and the strong increase of microbial N and P after fertilization is the first direct evidence of a high potential for nutrient immobilization in these soils. This observation supports the suggestions, from indirect observations, that the low recovery of added fertilizers in plant biomass could be due to nutrient immobilization by soil microorganisms (e.g. Shaver and Chapin 1980; Marion et al. 1982; Shaver et al. 1986). It also agrees with observations of low seasonal net nutrient mineralization in a variety of arctic soils (Chapin et al. 1988; Giblin et al. 1991; Nadelhoffer et al. 1991) including low and even negative net growing season N and P mineralization found on the same heath types situated near to our present sites (Jonasson et al. 1993).

Presumably, the retention of nutrients we found in microorganisms after fertilizer addition also takes place during naturally occurring flushes, e.g. those in connection with drying and wetting or freeze-thaw cycles (Smith and Paul 1990). This implies that microbial nutrient uptake could cause a considerable depletion of the inorganic nutrient pool that otherwise would have been available for plant uptake.

All responses we measured are integrated responses across all components (e.g. bacteria and fungi) and functional groups of the microbial community. It is possible therefore that certain groups of microorganisms responded in different ways to the additions. Although such differential responses are interesting in detailed studies of microbial turnover of organic matter and nutrients in the soil, they are less important in the context of this study which aimed primarily to examine responses to the additions as net nutrient partitioning between soil nutrient pools.

The strong immobilization of nutrients we found in the microbial biomass as a whole suggests that the microbes could act, at least periodically, as competitors with the plants for nutrients (Schimel et al. 1989; Harte and Kinzig 1993). The availability of inorganic nutrients to the plants may therefore depend heavily not only on the microbial mineralization rate of litter and soil organic matter, but also on the ability of the plants to compete with soil microorganisms, unless the plants can use pulses of nutrients released to the inorganic soil nutrient pool after die-back of the microbial community. Another way for plants to circumvent low nutrient availability, which is well known but so far poorly explored in the Arctic, is by forming symbiotic associations with the soil fungal community. For instance, the ericaceous plants that dominate on heaths are hosts to mycorrhizal fungi that can break down and utilize nitrogen-containing, complex organic compounds (Read 1991). Hence, nutrient fractions which are unavailable for most other plant groups may be assimilated by the ericaceous plants, and they thus obtain a competitive advantage over other plants, which would explain their dominance on nutrient deficient soils (Read 1991). Recent results indicate that organic N is indeed acquired by ectomycorrhizal and ericoid mycorrhizal dwarf shrubs in the subarctic (Michelsen et al. 1996). This is being explored further experimentally by assessing plant nutrient uptake with and without benomyl treatments.

Acknowledgements We are much indebted to Professor Mats Sonesson and the staff at the Abisko Scientific Research Station for logistic support and to Morten Heegard, Lotte Illeris, Cristina Pomar, Pernille Thorbeck and Ole Vestergaard for assistance in the field. The work was financed by the Danish Natural Science Research Council grants no. 11-0421-1, 11-0611-1, 9501046 and grant no. 127402 from the Swedish Environmental Protection Board. T. V. C. is grateful to the U. K. National Environment Research Council for financial support.

References

Brookes PC, Powlson DS, Jenkinson DS (1982) Measurement of microbial biomass phosphorus in soil. Soil Biol Biochem 14:319–329

Brookes PC, Kragt JF, Powlson DS, Jenkinson DS (1985a) Chloroform fumigation and the release of soil nitrogen: the effect of fumigation time and temperature. Soil Biol Biochem 17:831–835

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985b) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in the soil. Soil Biol Biochem 17:837–842

Chapin FS III, Barsdate RJ, Barel D (1978) Phosphorus cycling in Alaskan coastal tundra: a hypothesis for the regulation of nutrient cycling. Oikos 31:189–199

Chapin FS III, Fetcher N, Kielland K, Everett KR, Linkins AE (1988) Productivity and nutrient cycling of Alaskan tundra: enhancement by flowing soil water. Ecology 69:693–702

Cheng W, Virginia RA (1993) Measurements of microbial biomass in arctic tundra soils using fumigation-extraction and

substrate-induced respiration procedures. Soil Biol Biochem 25:135–141
Fitter AH (1986) Effect of benomyl on leaf phosphorus concentration in alpine grasslands: a test of mycorrhizal benefit. New Phytol 103:767–776
Fitter AH, Nichols R (1988) The use of benomyl to control infection by vesicular-arbuscular mycorrhizal fungi. New Phytol 110:201–206
Giblin AE, Nadelhoffer KJ, Shaver GR, Laundre JA, McKerrow AJ (1991) Biogeochemical diversity along a riverside toposequence in arctic Alaska. Ecol Monogr 61:415–435
Haag RW (1974) Nutrient limitations to plant production in two tundra communities. Can J Bot 52:103–116
Harte J, Kinzig AP (1993) Mutualism and competition between plants and decomposers: implications for nutrient allocation in ecosystems. Am Nat 141:829–846
Jenkinson DS, Powlson DS (1976) The effect of biocidal treatments on metabolism in soil -V. A method for measuring soil biomass. Soil Biol Biochem 8:209–213
Jonasson S (1992) Growth responses to fertilization and species removal in tundra related to community structure and clonality. Oikos 63:420–429
Jonasson S (in press) Buffering of arctic plant responses in a changing climate. In: Oechel WC, Callaghan TV, Gilmanov T, Holten JI, Maxwell B, Molau U, Sveinbjörnsson B (eds) Global change and arctic terrestrial ecosystems. Springer-Verlag
Jonasson S, Chapin FS III (1991) Seasonal uptake and allocation of phosphorus in *Eriophorum vaginatum* L. measured by labelling with ^{32}P. New Phytol 118:349–357
Jonasson S, Havström M, Jensen M, Callaghan TV (1993) In situ mineralization of nitrogen and phosphorus of arctic soils after perturbations simulating climate change. Oecologia 95:179–186
Kedrowski RA (1983) Extraction and analysis of nitrogen, phosphorus and carbon fractions in plant material. J Plant Nutr 6:989–1011
Koide RT, Huenneke LF, Hamburg SP, Mooney HA (1988) Effects of applications of fungicide, phosphorus and nitrogen on the structure and productivity of an annual serpentine plant community. Funct Ecol 2:335–344
Liu LX, Hsiang T (1994) Bioassays for benomyl adsorption and persistence in soil. Soil Biol Biochem 26:317–324
Marion GM, Miller PC, Kummerow J, Oechel WC (1982) Competition for nitrogen in a tussock tundra ecosystem. Plant Soil 66:317–327
Michelsen A, Schmidt IK, Jonasson S, Dighton J, Jones HE, Callaghan TV (1995) Inhibition of growth, and effects on nutrient uptake of arctic graminoids by leaf extracts – allelopathy or resource competition between plants and microbes? Oecologia 103:407–418
Michelsen A, Schmidt IK, Jonasson S, Quarmby C, Sleep D (1996) Leaf ^{15}N abundance of subarctic plants provides field evidence that ericoid, ectomycorrhizal and non- and arbuscular mycorrhizal species access different sources of soil nitrogen. Oecologia 105:53–63
Nadelhoffer KJ, Giblin AE, Shaver GR, Laundre JL (1991) Effects of temperature and substrate quality on element mineralization in six arctic soils. Ecology 72:242–253
Parinkina OM (1974) Bacterial production in tundra soils. In: Holding AJ, Heal OW, MacLean SF Jr, Flanagan PW (eds) Soil organisms and decomposition in tundra. Tundra Biome Steering Committee, Stockholm, pp 65–77
Patra DD, Brookes PC, Coleman K, Jenkinson DS (1990) Seasonal changes of soil microbial biomass in an arable and a grassland soil which have been under uniform management for many years. Soil Biol Biochem 22:739–742
Post WM (1993) Uncertainties in the terrestrial carbon cycle. In: Solomon AM, Shugart HH (eds) Vegetation dynamics and global change. Chapman and Hall, New York, pp 116–132
Read DJ (1991) Mycorrhizas in ecosystems – nature's response to the "law of minimum". In: Haksworth DL (ed) Frontiers in mycology. Honorary lectures from the fourth international mycological congress, Regensburg 1990. C.A.B. International, Wallingford, pp 101–130
Ross DJ, Tate KR (1993) Microbial C and N, and respiratory activity, in litter and soil of a southern beech (*Nothofagus*) forest: Distribution and properties. Soil Biol Biochem 25:477–483
Rosswall T (1976) The internal nitrogen cycle between microorganisms, vegetation and soil. In: Svensson BH, Söderlund R (eds) Nitrogen, phosphorus and sulphur – global cycles (SCOPE Report 7). Ecol Bull 22:157–167
Rosswall T, Flower-Ellis JGK, Johansson LG, Jonsson S, Rydén BE, Sonesson M (1975) Stordalen (Abisko) Sweden. In: Rosswall T, Heal OW (eds) Structure and function of tundra ecosystems. Ecol Bull 20:265–294
Sarathchandra SU, Perrott KW, Littler RA (1989) Soil microbial biomass: influence of simulated temperature changes on size, activity and nutrient-content. Soil Biol Biochem 21:987–993
Schimel JP, Jackson LE, Firestone MK (1989) Spatial and temporal effects on plant-microbial competition for inorganic nitrogen in a California annual grassland. Soil Biol Biochem 21:1059–1066
Shaver GR, Chapin FS III (1980) Response to fertilization by various plant growth forms in an Alaskan tundra: nutrient accumulation and growth. Ecology 61:662–675
Shaver GR, Chapin FS III (1995) Long-ferm responses to factorial NPK fertilizer treatment by Alaskan wet and moist fundra sedge species. Ecography 18:259–275
Shaver GR, Chapin FS III (1986) Effect of fertilizer on production and biomass of tussock tundra, Alaska, U.S.A. Arct Alp Res 18:261–268
Shaver GR, Chapin FS III, Gartner BL (1986) Factors limiting seasonal growth and peak biomass accumulation in *Eriophorum vaginatum* in an Alaskan tussock tundra. J Ecol 74:257–278
Smith JL, Paul EA (1990) The significance of soil microbial biomass estimations. In: Bollag JM, Stotsky G (eds) Soil biochemistry, vol 6. Marcel Dekker, New York, pp 357–396
Sparling GP, Feltham CV, Reynolds J, West AW, Singleton P (1990) Estimation of soil microbial C by a fumigation-extraction method: use on soils of high organic matter content, and a reassessment of the k_{EC}-factor. Soil Biol. Biochem 22:301–307
Tate KR, Ross DJ, Feltham CW (1988) A direct extraction method to estimate soil microbial C: effects of experimental variables and some different calibration procedures. Soil Biol Biochem 20: 329–335
Ulrich A, Gersper PL (1978) Plant nutrient limitations of tundra plant growth. In: Tieszen LL (ed) Vegetation and production ecology of an Alaskan arctic tundra. Springer, Berlin Heidelberg New York, pp 457–482
Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707
Voroney RP, Winter JP, Beyaert RP (1993) Soil microbial biomass C and N. In: Carter MR (ed) Soil sampling and methods of analysis. Lewis, Boca Raton, pp 277–286
Warren Wilson J (1966) An analysis of plant growth and its control in arctic environments. Ann Bot 30:383–402
Williams BL, Sparling GP (1984) Extractable N and P in relation to microbial biomass. Plant Soil 76:139–148
Wood T, Bormann FH, Voigt GK (1984) Phosphorus cycling in a northern hardwood forest: biological and chemical control. Science 223:391–393
Zak DR, Groffman PM, Pregitzer KS, Christensen S, Tiedje JM (1990) The vernal dam: plant-microbe competition for nitrogen in northern hardwood forests. Ecology 71:651–656