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Impacts of chronic nitrogen additions vary seasonally and by microbial functional group in tundra soils

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Abstract. Previous studies have shown that fertilization with nitrogen depresses overall microbial biomass and activity in soil. In the present study we broaden our understanding of this phenomenon by studying the seasonality of responses of specific microbial functional groups to chronic nitrogen additions in alpine tundra soils. We measured soil enzyme activities, mineralization kinetics for 8 substrates, biomass of 8 microbial functional groups, and changes in N and carbon pools in the soil. Our approach allowed us to compare the ability of the soil microbial biomass to utilize various substrates in addition to allowing us to estimate changes in biomass of microbial functional groups that are involved in carbon and nitrogen cycling. Overall microbial activity and biomass was reduced in fertilized plots, whereas pools of N in the soil and microbial biomass N were higher in fertilized plots. The negative effects of N were most prominent in the summer. Biomass of the dominant microbial functional groups recovered in fertilized soils during the winter and nitrogen storage in microbial biomass was higher in fertilized soils in the autumn and winter than in the summer. Microbial immobilization of N may therefore be a significant sink for added N during autumn and winter months when plants are not active. One large microbial group that did not recover in the winter in fertilized soils was phenol mineralizers, possibly indicating selection against microbes with enzyme systems for the breakdown of phenolic compounds and complex soil organic matter. Overall, this work is a step towards understanding how chronic N additions affect the structure and biogeochemical functioning of soil microbial communities.

Introduction

The impacts of nitrogen (N) deposition on the function of ecosystems are of global interest and a comprehensive understanding requires more knowledge of the response of soil microorganisms to N additions. The processing of incoming N by soil microorganisms can be an important avenue for retention of N in soil (Fisk et al. 1998; Stark and Hart 1997; Zogg et al. 2000) and it is possible that this processing is more important in ecosystems impacted by deposition (Fisk and Schmidt 1996).

The alpine tundra of the Rocky Mountains is an ecosystem that, because of a short growing season and cold temperatures, may be particularly sensitive to the

deposition of N. Current yearly rates of N-deposition are about 6 kg N ha^{-1} (Sievering 2001) but with the recent acceleration in the urbanization of the Rocky Mountain region, deposition of nitrogenous compounds is increasing (Williams et al. 1996; Williams and Caine 2001) and high mountain areas may be receiving a disproportionate amount of these pollutants (Burns 2002). In particular, the high-elevation areas of the Colorado Front Range are receiving increased amounts of pollutants (mainly HNO_3, NO_3^-, NH_4^+ and NH_3) from power plant emissions in Western Colorado and from sources as far away as Southern California and Northern Mexico (Sievering 2001). The Denver metropolitan area also contributes to nitrogen (N) loading of alpine areas during easterly (upslope) storms, especially in the summer (Burns 2002; Parrish et al. 1986; Williams et al. 1996). Despite this increasing rate of N deposition, we know very little about the effects of these pollutants on high elevation ecosystems of the Rocky Mountains (Bowman et al. 1995; Williams et al. 1996).

Especially understudied are the effects of N-deposition on microbial communities and microbial biogeochemistry in high mountain systems. Previous work on Niwot Ridge indicated that the microbial community of alpine tundra was capable of immobilizing added N in amounts equivalent to several times the present rate of anthropogenic N inputs to the system (Fisk and Schmidt 1996). However, this effect was only seen in the fall. Increased N loading causes other microbial processes to change in more negative ways. For example, nitrous oxide emissions increased and methane oxidation decreased significantly in response to N fertilization of alpine tundra soils (Neff et al. 1994). In order to understand the underlying mechanism of these microbial biogeochemical responses, more detailed studies of how N deposition affects microbial community dynamics is needed.

In the present study we investigated the effects of N-deposition on the alpine tundra microbial community and its functions in various C and N transformations. We quantified microbial biomass as a whole and also as functional groups capable of mineralizing different C substrates. We also measured soil enzyme activities, mineralization kinetics, and changes in N and C pools in the soil. Our approach allowed us to compare the ability of the soil microbial biomass to mineralize various substrates in addition to allowing us to estimate changes in population sizes of microbial functional groups that are involved in carbon and N cycling. This work is a major step towards understanding how chronic N additions affect the structure and functioning of soil microbial communities.

Methods

The research was conducted on Niwot Ridge, a large expanse of alpine tundra located just east of the continental divide of the Front Range of the Rocky Mountains (40°03′ N, 105°36′ W). The ″dry meadow″ tundra sites used in this study have been described in detail elsewhere (Seastedt and Vaccaro 2001). Five fertilized and five control plots (2 × 2 m) were established in the autumn of 1993. Nitrogen additions

averaged 10 g N m^{-2} y^{-1} over the six-year duration of this study. Nitrogen amendments consisted of a mixture of ammonium nitrate (NH_4NO_3) and ammonium sulfate (NH_4SO_4). In September 1993 and August 1994, 20 g N m^{-2} was added as NH_4NO_3 to each of the fertilized plots and no fertilizer was added in 1995. In 1996 and 1997, 10 g N m^{-2} was applied as NH_4SO_4 in July of each year. No N was added in 1998 before our February 1998 samples were collected.

Soils were sampled to a depth of 10 cm with a soil corer (2.5 cm. diameter). Because we wanted to minimize impacts of sampling, soils from 3 randomly chosen cores from each plot were pooled at each sampling date. Soils were coarsely sieved (4.75 mm mesh size) to remove rocks and large roots and were kept at 4 °C until they were processed in the laboratory (less than 48 hours).

Soil nitrogen pools

Proteins and amino acids were extracted from soil using 0.1 mol/L bicarbonate ($NaHCO_3$) and deionized water, respectively, as described by Lipson et al. (1999b). Solutions were added in a ratio of 5 mL per g of soil and mixtures were shaken for 30 minutes and filtered through Whatman number 3 filter papers. Amino acid, protein, and ammonium concentrations were measured as described earlier (Lipson et al. 1999b). Ammonium was also measured in the K_2SO_4 extracts on some dates as described previously (Lipson et al. 1999b).

Microlysimeters (Rhizosphere Research Products, Wageningen, Netherlands) were installed to a depth of 10 cm at least 1 month before sampling was started. Sampling and solution analyses were performed as described by Raab et al. (1999).

Soil enzyme measurements

Protease activities were measured as described by Lipson et al. (1999b) using a modification of the method of Watanabe and Hayano (1995). Briefly, soils were incubated with and without casein to measure potential and ″native″ protease rates, respectively. Soils were incubated at a temperature of 5 °C for six hours, during which time rates were linear.

Cellulase activities were measured as described by Lipson et al. (2002). Carboxymethylcellulose was added (0.5% final concentration) with citrate buffer (50 mM, pH 6) and toluene (0.2 mL) to 5 g of soil to obtain a final volume of 10 mL. Tubes were shaken at 22 °C for 24 h, centrifuged, and the glucose released was measured using a reducing sugar assay (Lipson et al. 2002; Schinner and von Mersi 1990).

Potential rates of carbon substrate use were estimated by incubating soil samples with various carbon substrates (acetate, glucose, glutamate, glycine, phenol, salicylate, 2,4-dinitrophenol and pentachlorophenol) and measuring kinetics of CO_2 production from those substrates as described below.

Microbial biomass and kinetic measurements

Biomass of different microbial functional groups was estimated by using the substrate-induced growth-response (SIGR) method or the substrate-induced respiration (SIR) method. Both methods are based on the observation that the initial rate of CO_2 production from a specific substrate is proportional to the biomass of microorganisms that can mineralize that substrate. These methods have been validated by direct comparison to other methods in studies of tundra and other soils (Colores et al. 1996; Lipson et al. 1999b; Schmidt 1992; Schmidt et al. 2000). Freshly collected soils (10 g dry wt. equivalent) were incubated in biometer flasks (Schmidt and Scow 2001) with substrates added at concentrations that were previously determined to induce maximum rates of substrate usage or growth of specific microbial functional groups. In the present study SIGR estimates of biomass levels were used when the added substrate induced the growth of the standing biomass that could utilize a specific substrate. In instances when the amount of substrate was insufficient to induce growth, estimates were obtained using the SIR approach. The concentrations of substrates used for the SIR and SIGR assays were 500 μg C g soil^{-1} for acetate, 2000 μg C g^{-1} for glutamate, 400 μg C g^{-1} for glycine, 50 μg C g^{-1} for phenol, 200 μg C g^{-1} for salicylate, 4 or 10 μg C g^{-1} for 2,4-dinitrophenol and 10,000 or 2000 μg C g^{-1} for glucose in 1996 and 1997, respectively. A small amount of ^{14}C-labeled substrate (approx. 20,000 dpm g soil^{-1}) was also added as a tracer of CO_2 production from the substrate. Carbon dioxide emitted from the soil was captured in 0.5 N NaOH in the sidearm of the biometer flask. At regular intervals, samples were removed with a syringe and replaced with fresh NaOH solution.

The increase in the rate of mineralization during the initial phase of incubation (as in Figures 1 and 2) was used to estimate the initial biomass level and the maximum specific growth rate of that biomass by fitting the SIGR equation (Colores et al. 1996) to the accelerating portions of the data:

$$dP/dt = \mu X_1 e^{\mu t} \tag{1}$$

in which X_1 is microbial biomass in terms of CO_2 produced, P is CO_2-carbon and μ is the maximum specific growth rate of CO_2 evolution during the incubation. To convert X_1 (with units of μg CO_2-C/g soil) to X_0 (μg biomass-C/g soil) we used the relationship of Colores et al. (1996):

$$X_0 = X_1(Y_c/(1 - Y_c)) \tag{2}$$

where Y_c is the yield coefficient with units of μg biomass-C/μg substrate-C.

Y_c was estimated from accumulated CO_2 data (as in Figure 3) using non-linear regression and a modification of the integrated logistic equation (Fisk 1995):

$$P = S_0 - (S_0 Y_c + (1 - Y_c)((S_0 + X_1)((S_0 e^{-\mu t})/(X_1 + S_0 e^{-\mu t})))) \tag{3}$$

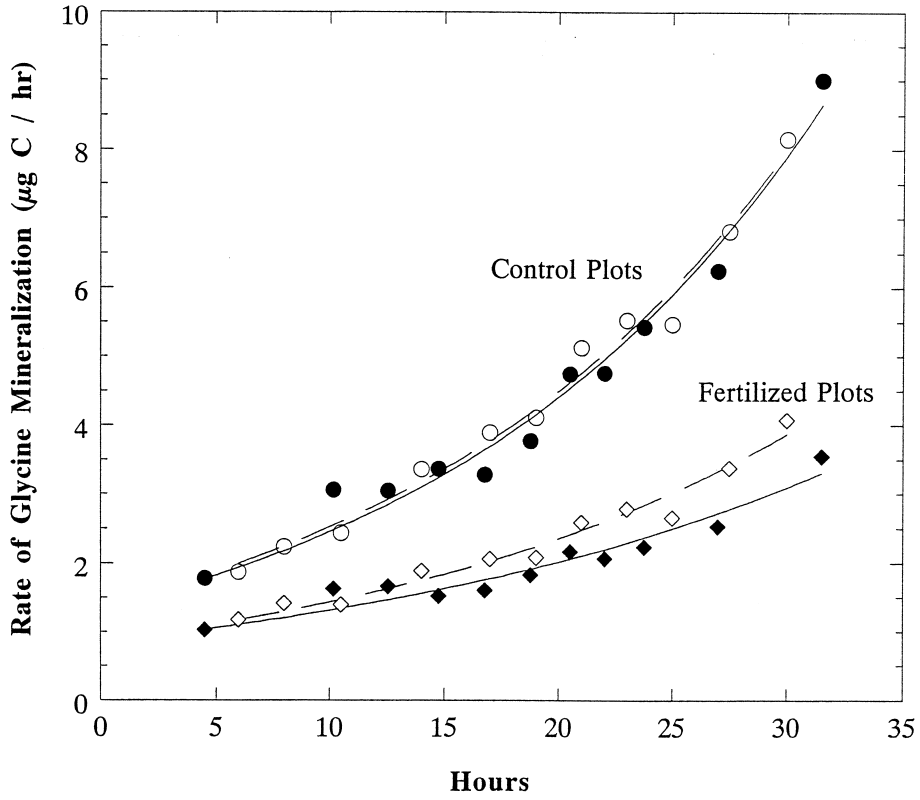

Figure 1. Change in rate of glycine mineralization during the accelerating portion of SIGR curves for fertilized (diamonds) and control (circles) soils collected before fertilization that year (June; closed symbols) and after fertilization (July; open symbols) in 1996. The lines are non-linear fits of Equation 1 to the data. For the control soils model SIGR parameter estimates were $\mu = 0.058$ and 0.059, $X_1 = 24.5$, and 23.3 and $r^2 = 0.985$ and 0.975. For the fertilized soil $\mu = 0.050$ and 0.043, $X_1 = 17.6$ and 19.9 and $r^2 = 0.968$ and 0.929, for June and July respectively.

where P is accumulated CO_2 production, S_0 is the initial substrate concentration and all other parameters are as described above. Equation 3 was fit to curves of accumulated CO_2 and estimates Y_c as the asymptote of the logistic-shaped respiration curves.

On several dates microbial C and N were also quantified using the chloroform fumigation-extraction (CFE) as described by Lipson et al. (1999b) except that no correction factor (K_c) was used to estimate microbial C.

Statistical analyses

The data were analyzed using two-way ANOVAs with fertilization and date as categorical variables, except for the glycine data, which were analyzed with a one-way ANOVA because only one sample was collected on each date. On some dates

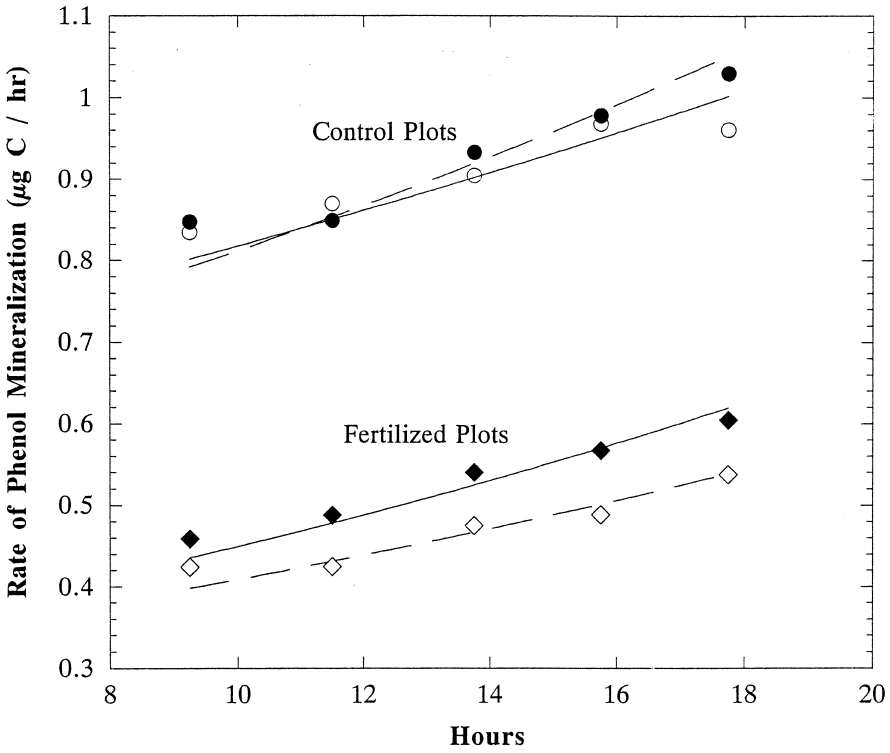

Figure 2. Change in rate of phenol mineralization in replicate control (circles) and fertilized (diamonds) soil samples collected in October 1997. The lines are non-linear fits of Equation 1 to the replicate sets of data. For the control curves $\mu = 0.018$ and 0.025, $X_1 = 38.7$ and 25.9, and $r^2 = 0.929$ and 0.955. For the fertilized soils $\mu = 0.033$ and 0.030, $X_1 = 10.3$ and 10.6, and $r^2 = 0.992$ and 0.935.

too little soil was obtained from each plot to run extensive tests and therefore samples were combined. This pooling of soils also allowed us to minimize disturbance to these long-term plots. Thus, while our statistical tests do not address within plot spatial variability, the means are essentially spatial averages, and the statistics show treatment effects are greater than experimental error. Given that this research was done at only one site, the results cannot be extrapolated to fertilizer effects in general.

Results

Nitrogen pools

Inorganic nitrogen concentrations were higher in fertilized plots in both 1996 and 1997 and inorganic N recovered in micro-lysimeters was also much higher in fertilized soils (Table 1). Organic nitrogen pools also were affected by fertilization.

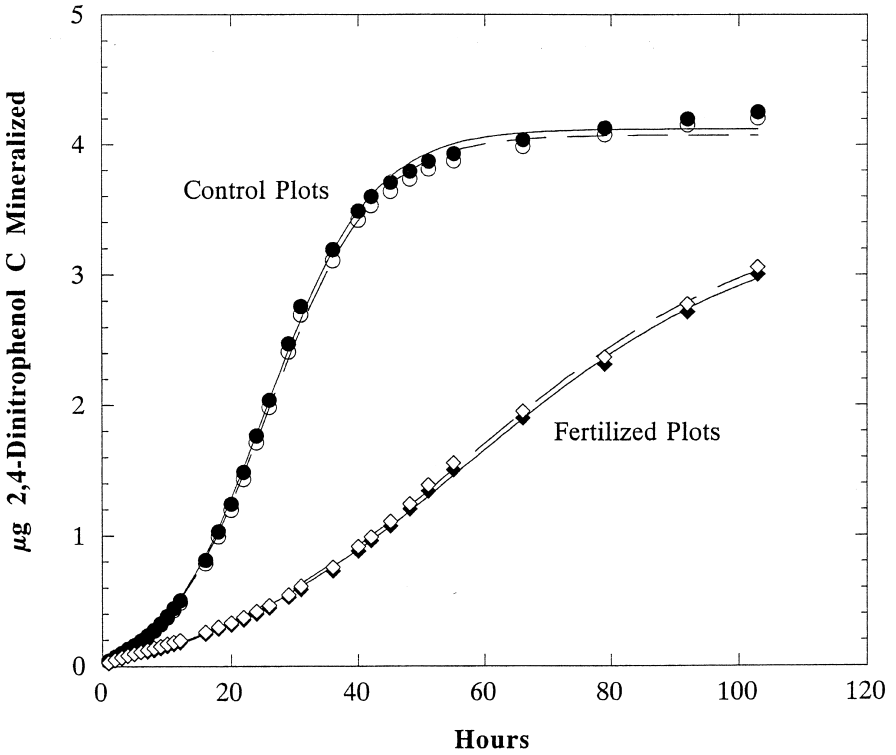

Figure 3. Curves of CO_2 accumulation from 2,4-dinitrophenol mineralization in fertilized (diamonds) and control (circles) soil samples collected in June 1996. The lines are fits of Equation 3 to the data. For the control curves model (Equation 3) parameter estimates were Y_c = 0.592 and 0.588. For the fertilized curves Y_c = 0.648 and 0.654.

Total soil protein levels were always higher in fertilized plots, but microbial biomass N was not significantly higher in fertilized plots (Table 2). Rates of proteolysis tended to be lower in fertilized soils, although maximal potential rates (+ casein) were not significantly higher in control soils (Table 3). The increase in protein (Table 2) on 6/17/97 did not result in an increase in the rate of proteolysis (Native rate, 6/17/97, Table 3) which may mean that the increase in protein was not available to the proteolytic microbial biomass.

Kinetics of substrate mineralization in soil

Nitrogen fertilization had a significant effect on the ability of alpine soils to mineralize a variety of organic compounds. In addition to the slight effect on proteolysis noted above, rates of cellulase activity were much lower in fertilized soils (Table 3).

We also compared the kinetics of substrate utilization in control versus fertilized soils. A useful way to assess the impact of N-fertilization on soils is to assess the

Table 1. Inorganic nitrogen in soil (to 10 cm depth) and micro-lysimeters soil water samples from control and fertilized plots. For comparison, Fisk and Schmidt (1996) reported total soil N of 7.4 (0.6) and 9.4 (0.8) mg g^{-1} for control and fertilized plots respectively. Standard errors of the mean are in parentheses (n = 5).

	Soil N		Lysimeter N	
	NO_3^- (μg N g^{-1})	NH_4^+ (μg N g^{-1})	NO_3^- (μg N mL^{-1})	NH_4^+ (μg N mL^{-1})
6/12/96				
Con.	2.1 (0.4)	nd*	nd	nd
+ N	15.7 (1.9)	nd	nd	nd
6/17/97				
Con.	0	16.7 (0.4)	1.5 (0.1)	0.30 (0.04)
+ N	14.7 (0.3)	95.9 (1.1)	10.5 (2.7)	9.0 (1.4)

*nd = not determined

Table 2. Organic nitrogen and microbial-biomass carbon (MBC) and nitrogen (MBN) in soil from control and fertilized plots. Standard errors of the mean are in parentheses. Values in any column followed by different letters were significantly different ($P < 0.01$). For comparison, Fisk and Schmidt (1996) reported total soil N of 7.4 (0.6) and 9.4 (0.8) mg g^{-1} for control and fertilized plots, respectively.

	Soil Protein $NaHCO_3$ ext. (μg g^{-1})	MBN μg N g^{-1}	MBC μg C g^{-1}	C:N[a]	Ext[b] N μg N g^{-1}	Ext. C μg C g^{-1}
6/17/97 (n = 3)						
Con.	5750 (750)a	nd[c]	nd	nd	nd	nd
+ N	8417 (300)b	nd	nd	nd	nd	nd
10/5/97 (n = 3)						
Con.	5023 (359)a	79 (11)a	658 (89)a	8.3a	58 (0.5)a	91 (15)a
+ N	6735 (618)b	95 (14)a	477 (36)a	5.0b	156 (4.3)b	132 (27)a

1. [a] C-to-N ratio of the microbial biomass
2. [b] Ext. = K_2SO_4 extracts
3. [c] nd = not done

relative ability of fertilized soils to mineralize various substrates. We tested both naturally occurring substrates (glucose, glutamate, glycine, salicylate, and phenol) and xenobiotic substrates (2,4-dinitrophenol and pentachlorophenol). As one example of how fertilization affected kinetics of substrate utilization, Figure 1 shows the time course of rate increases for fertilized and control soils incubated with glycine (400 μg C g^{-1}). Note that the rate increases exponentially in each soil, but that the initial rate is lower in the fertilized soil and the rate of change in the rate is different for the two soils. This was the most common pattern seen during this study and these types of curves were used to estimate the biomass levels of microbial functional groups for fertilized and control soils (see next section).

Table 3. Protease and cellulase rates in soil from control and fertilized plots. Standard errors of the mean are in parentheses (n = 3 for 6/12/96 and n = 2 for 6/17/97). Cellulase rates were significantly different between control and fertilized soils ($P < 0.0001$), whereas protease rates were not different except for the Native rates on 6/12/96 ($P < 0.05$).

	Max. Protease Rates		
	Native (nmol g^{-1} h^{-1})	+ Casein (nmol g^{-1} h^{-1})	Cellulase Rates (μmol g^{-1} h^{-1})
6/12/96			
Con.	47 (1.4)	60 (11)	nd
+ N	33 (1.1)	47 (11)	nd
6/17/97			
Con.	15 (2.1)	62 (3.8)	1.65 (0.027)
+ N	12 (11.6)	52 (8.0)	0.74 (0.030)

Even more dramatic than the effects of fertilization on amino acid utilization was the effect on the kinetics of phenol mineralization. Figure 2 shows results from an autumn incubation of soils from fertilized and control plots. The largest effect was on the initial rate of phenol mineralization, which was twice as high in the control soils as in the fertilized soils (Figure 2).

The ability of soils to mineralize xenobiotic phenolic compounds such as 2,4-dinitrophenol (DNP) (Figure 3) and pentachlorophenol (PCP) (data not shown) was also significantly compromised by N fertilization. Figure 3 shows curves of accumulated CO_2 from 2,4-dinitrophenol from 2 fertilized and control plots on one sampling date. The negative kinetic shift caused by fertilization may be the result of a decreased population of 2,4-dinitrophenol users (see below). Data such as those depicted in Figure 3 were used to estimate Y_c (see below).

Biomass of microbial functional groups

In addition to several measurements of microbial biomass C and N (Table 2) our main approach to studying microbial biomass dynamics was to use the SIR and SIGR approaches that have been previously shown to work extremely well in acidic alpine soils (Colores et al. 1996; Lipson et al. 1999b).

Total microbial biomass

Overall, levels of microbial biomass were lower in soils from fertilized plots than in soil from control plots. The best substrates for estimating total microbial biomass levels in alpine soils are glucose and glutamate (Colores et al. 1996; Lipson et al. 1999b). The biomass that could utilize glucose was higher than that which could use glutamate, but the use of both substrates independently demonstrated that fertilization significantly decreased microbial biomass during the summer in both years of this study (compare Figure 4 and Table 4). In both fertilized and control plots, glutamate mineralizers increased dramatically in the autumn and winter and

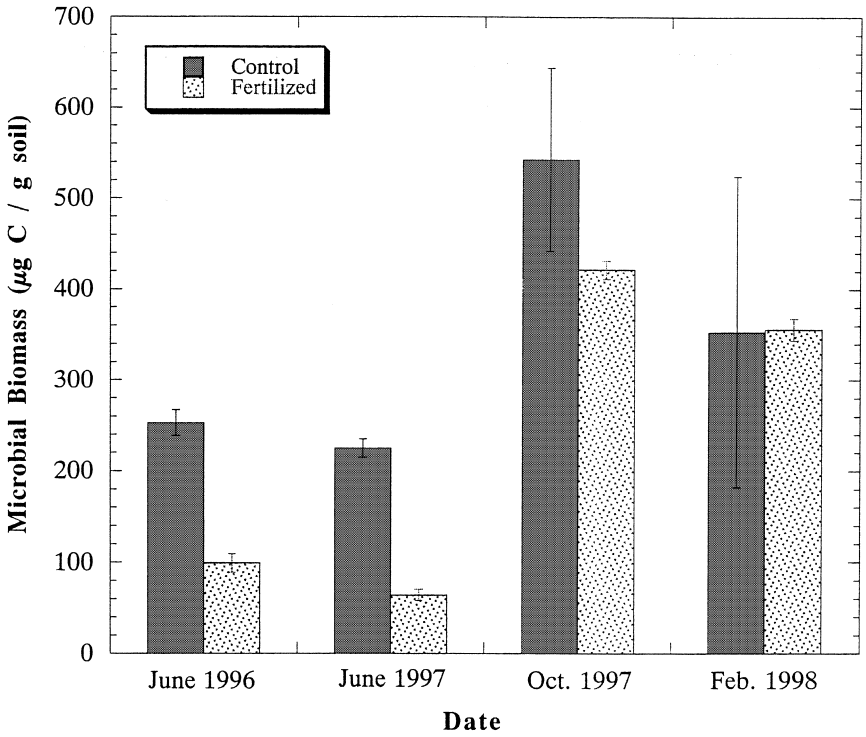

Figure 4. Seasonal estimates of microbial biomass (glutamate mineralizers) in fertilized and control plots. Overall there was a significant effect of date (p = 0.004) and a marginally significant effect of fertilizer treatment (p = 0.062). The effects shown are residual effects since the fertilizer additions for 1996 and 1997 were in July, i.e., after soil samples were taken.

the difference between fertilized and control soils was less in the autumn and winter than during the two summer samplings (Figure 4). In fact there was no significant difference between the two soils in February 1998.

To increase confidence in our SIGR biomass estimates we also estimated microbial biomass using the chloroform fumigation (CF) method on one of our sampling dates. Microbial biomass C estimates from the chloroform fumigation method (658 and 477 μg C g^{-1}; Table 2) were similar to SIGR biomass C estimates (543 and 422 μg C g^{-1}; Figure 4) obtained on the same day (10/5/97). In addition, Lipson et al. (1999b) found a highly significant (P = 0.0001, n = 22–28) correlation between year-round SIGR and CF biomass estimates.

We also estimated the biomass of microbes that could use acetate and glycine, which are both simple compounds found in alpine soils and have been shown to be used by smaller microbial populations than either glutamate or glucose in alpine soils (Lipson et al. 1999a; West and Schmidt 1999). In the presnt study, the biomass that could utilize these substrates was about an order of magnitude lower than the biomass of glucose and glutamate mineralizers, and there was a significant de-

Table 4. Biomass estimates of microbial functional groups that mineralize different substrates in tundra soils. Controls for any substrate on a given date (column) are significantly different from fertilized values if they are followed by one or more asterisks (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). There were also significant effects of date ($P < 0.05$) for the glucose and salicylate data. Summer samples were taken in June except for glycine and 2,4-DNP, which are means of samples taken in June and July.

Substrate	treatment	Biomass (μg C g^{-1})		
		Summer 1996	Summer 1997	Oct. 1997
Glucose	Control	547. (28)**	645. (111)**	nd
(n = 3, 2)	+ N	243. (37)	452. (59)	nd
Acetate	Control	.nd	6.9 (2.3)***	nd
(n = 2)	+ N	.nd	1.1 (0.1)	nd
Glycine	Control	15.7 (1.4)**	.nd	nd
(n = 2)	+ N	9.4 (1.0)	.nd	nd
2,4-DNP	Control	0.35 (0.07)*	.nd	nd
(n = 5)	+ N	0.18 (0.06)	.nd	nd
Salicylate	Control	0.32 (0.02)	0.34 (0.01)	0.41 (0.06)***
(n = 2, 2, 2)	+ N	0.30 (0.01)	0.34 (0.001)	0.78 (0.05)

pression in the guild of microbes that utilizes these substrates in the fertilized soils (Table 4).

Phenolic mineralizers

In general, fertilization had a negative effect on populations of organisms that could mineralize phenolic substrates (phenol, salicylate and 2,4-dinitrophenol). The largest group of phenolic-mineralizers in our soils were those that could mineralize phenol. This group was significantly lower in fertilized plots in both years of this study (Figure 5) and did not show the winter-time convergence with control biomass levels noted above for glutamate mineralizers (Figure 4). Microbial populations that could utilize a xenobiotic phenolic compound, 2,4-dinitrophenol (DNP), were also lower in fertilized plots (Table 4). In contrast, populations that could mineralize salicylate were not affected by fertilization in the summer and biomass of salicylate mineralizers in fertilized soils actually surpassed biomass levels in control plots in the fall of 1997 (Table 4). It should be noted, however, that the biomass of salicylate and DNP mineralizers were almost 2 orders of magnitude lower than the population that could mineralize phenol (compare Figure 5 and Table 4).

Another general difference between fertilized and control soils was that yield coefficients for microbes in fertilized soils were generally lower than in control soils (Table 5). These differences are partially responsible for the lower production of biomass in fertilized soils noted above, but are not different enough to completely explain the lower biomass estimates noted for fertilized soils.

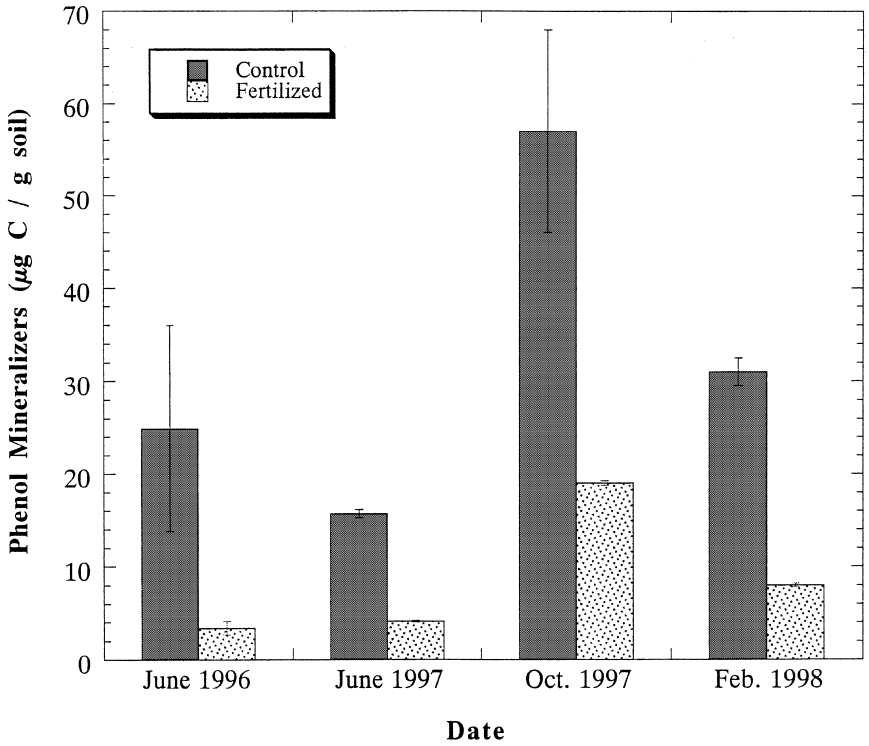

Figure 5. Seasonal estimates of microbial biomass capable of mineralizing phenol. Overall there was a significant effect of date (p = 0.0049) and a significant effect of fertilization (p = 0.0003).

Table 5. Yield (Y_c) estimates (microbial biomass C formed / C added) obtained by fitting Equation 3 to accumulated CO_2 curves for various substrates added to fertilized and control soils. These values were used in Equation 2 to estimate biomass of organisms capable of metabolizing each substrate. Standard errors are in parentheses.

Substrate	Y_c	
	Control Soil	Fertilized Soil
Glucose (n = 2)	0.66 (0.001)	0.60 (0.005)
Glutamate (n = 7)	0.44 (0.007)	0.43 (0.011)
Glycine (n = 2)	0.39 (0.018)	0.33 (0.005)
Phenol (n = 8)	0.67 (0.009)	0.63 (0.007)
Salicylate (n = 6)	0.08 (0.008)	0.08 (0.009)
2,4-dinitrophenol (n = 5)	0.61 (0.001)	0.62 (0.016)

Discussion

Microbial biomass and activity has been shown to be significantly suppressed by mid- to long-term (> 1 year) addition of nitrogen in a number of different studies

(Fisk and Fahey 2001; Prescott et al. 1992; Söderström et al. 1983). Our data for alpine tundra concur with this general pattern and extend the findings of Fisk (1995) who showed a significant decrease in microbial biomass in urea fertilized plots (Bowman et al. 1995) near our current study site. Our data build on the work of Fisk (1995) to show that some aspects of microbial function in decomposition decline in response to fertilization. Reduction of biomass of organisms capable of growth on glucose, acetate and amino acids suggests that the metabolism of these types of simple C sources is slowed in fertilized soils. Furthermore, reduction of cellulase activity in soils implies that decomposition of plant structural material is slowed by fertilization. The combination of lower biomass, reduced enzymatic activity, and slower kinetics of substrate utilization suggests that a reduction in overall decomposition processes may result from continued chronic additions of N to this alpine ecosystem. These results could explain why fertilization suppressed long-term litter decay rates in alpine tundra (Seastedt et al. 2001).

The question still remains as to why microbial activity and biomass would be reduced in response to fertilization. It is very difficult to attribute all of the effects seen in this study solely to nitrogen availability. Nitrogen fertilization not only increases levels of nitrogen in the soil but also can decrease soil pH, even in already acidic soils (Aerts and de Caluwe 1999; Fisk 1995; Fisk and Schmidt 1996). This was the case in the present study because the pH averaged 4.7 in plots receiving N compared to 5.3 for control plots (Seastedt and Vaccaro 2001). It is not clear if this pH change alone could be the cause of the shifts in microbial function noted in this study, and results from other soils are somewhat mixed in this regard. Thus, it can only be concluded that N fertilization has an overall depressive effect on many microbial functional groups, but the actual cause of this effect remains to be elucidated.

Seasonality of plant and microbial processes is pronounced in the alpine tundra (Brooks et al. 1998; Lipson et al. 1999b), with important implications for responses to N additions. The depression of microbial activity and biomass was especially pronounced in the summer when all estimates of total microbial biomass (chloroform-labile C, glucose and glutamate mineralizers) were lower in the fertilized plots. In contrast, the effect of fertilization on total microbial biomass was not as pronounced in the winter, despite the fact that it had been less time since the last fertilization event. In fact, total microbial biomass under the snow in February was about the same in the fertilized and control soils. The larger effect of fertilization in the summer than in the winter is consistent with our recent findings that there is a profound microbial community shift from summer to winter in tundra soils (Lipson et al. 2002, 1999b). Conditions under alpine snow packs are very conducive to microbial growth (Brooks et al. 1998; Lipson et al. 1999b) and the highest populations of microbes occur in the winter in alpine tundra soils (Lipson et al. 1999b). Microbes under the snow pack are adapted to the steady low temperatures (Lipson et al. 2000) and exist in wet soil containing high levels of available N and C, even in the absence of N fertilization (Brooks et al. 1998; Lipson et al. 1999b). It is therefore not surprising that the winter-adapted microbial community is less adversely affected by N fertilization than the summer-adapted community.

Another reason that the microbial biomass is not as adversely affected in the winter is that it is during this time that excess carbon is available in alpine soils (Lipson et al. 2000). This carbon excess is the result of plant senescence and can actually lead to the microbial biomass being nitrogen limited, at least for short periods of time. As a result microbial biomass levels can actually respond positively to N additions in the autumn but not in the summer (Fisk and Schmidt 1996). It should be remembered, however, that the make up of the biomass is probably quite different in fertilized soils even if the overall biomass level is not different from control soils. This is especially evident for microbes specializing in phenol degradation as discussed below.

The negative effects of fertilization were evident not only in the general heterotrophic microbial community (glucose and glutamate mineralizers) but also in more specialized microbial groups such as organisms capable of mineralizing phenolic compounds. In fact, the most profound negative effect in this study was on the large guild of microbes that could mineralize the simplest phenolic compound, phenol. Work at nearby talus sites indicates that phenol is metabolized mostly by bacteria (Ley and Schmidt 2002). If that is also the case at our sites, then the depression of phenol mineralizers may indicate a disproportionate suppression of bacteria by N fertilization (although alternative hypotheses are possible, as discussed below). This hypothesis is also supported by the fact that total microbial biomass was not suppressed in the winter by fertilization, when the microbial community at these sites is dominated by fungi (Lipson et al. 2002). Similarly, research on acidic (pH 4.6) grassland soils of North Wales has shown that N fertilization increases the ratio of fungal to bacterial fatty acids (Bardgett et al. 1999).

The decrease in phenol and 2,4-dintrophenol mineralization is partially explainable by the overall decrease in microbial biomass at most sampling dates. But the very pronounced suppression of phenol mineralization, and the fact that this suppression was not alleviated in the winter, may indicate an additional mechanism of suppression, such as selection against organisms with enzyme systems for degradation of phenolic compounds. In this regard, Carreiro et al. (2000) reported that added NH_4NO_3 substantially decreased phenol oxidase activity in soils containing oak litter. It is also possible that the increased level of ammonium in the fertilized soils is interfering with oxygenases that are involved in both phenol and methane oxidation by methane-oxidizing bacteria. We have previously shown that fertilization significantly reduced the oxidation of methane by alpine dry meadow soils (Neff et al. 1994), although this effect could also be explained by ammonia being a competitive inhibitor of methane utilization.

In contrast to the effects of fertilization on phenol and 2,4-dinitrophenol mineralization, salicylate mineralization was not adversely affected by fertilization (Table 4). Salicylate is a naturally occurring phenolic compound that is produced in high quantities by nearby willows (*Salix brachycarpa*) but is not found in abundance in the *K. myosuroides* plants that dominate the dry meadow sites of this study (Schmidt et al. 2000). Salicylate also does not support much microbial growth in these soils as indicated by the extraordinarily low biomass and Y_c values observed for salicylate. The low Y_c value (0.08) also indicate that salicylate is being metabo-

lized almost exclusively as an energy source because 92% of the carbon in salicylate was released as CO_2. This may indicate that salicylate is being used as a cometabolite with some unknown carbon source in these soils. The importance of salicylate mineralization in this soil is also questionable because the biomass that can mineralize salicylate is 2 orders of magnitude lower than the population that can mineralize phenol and almost 2 orders of magnitude lower than the biomass of salicylate mineralizers in soil from under nearby willow plants (Schmidt et al. 2000).

Our results have implications for understanding the ability of the microbial biomass to absorb future increases in N deposition to alpine tundra. Although the increase in microbial biomass N (MBN) was not significant in fertilized soils, the increase in soil protein and decrease in C-to-N ratio was significant (Table 2) indicating an increased capacity to immobilize N in fertilized soils despite the general decrease in soil microbial biomass. The C:N ratio of the chloroform-labile microbial biomass was 8.3 in the control plots and 5.0 in the fertilized plots. These values were very close to the C:N values estimated by Fisk (1995) in an earlier N-fertilization experiment done near our present site. Both studies point to at least a short-term ability of the microbial biomass to store excess N in tundra soils, especially in the autumn and winter. It is unlikely, however, that this could be a long-term sink for excess N given that fertilization causes a decrease in microbial biomass in these soils during the summer. A more likely long-term storage mechanism would be microbial transformation of excess N into soil organic matter as has already been indicated at these sites (Fisk and Schmidt 1996; Fisk et al. 1998).

Taken together, the results of this study show that both carbon and nitrogen cycling are affected by N fertilization at our alpine tundra sites. Our data indicate that fertilization not only depresses the biomass of organisms that utilize many substrates, but also depresses the rate at which the biomass responds to carbon inputs. It is encouraging, however, that during the winter, when N is most available in these soils (Brooks et al. 1998; Lipson et al. 1999b), the microbial biomass is least depressed by N fertilization. More work is needed to determine if the increased capacity of microbes to immobilize N in the winter and during snowmelt can prevent increased N loading to spring runoff. This is a critically important unanswered question given the increasing rate of population growth and resultant increases in N deposition occurring in the Rocky Mountain West of the United States.

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