Substrate limitations to microbial activity in taiga forest floors

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Abstract

A combination of laboratory and field experiments showed substantial differences in microbial activity, substrate processing, and N cycling in forest floor samples from different Alaskan boreal forest ecosystems. In soils from black spruce (Picea mariana [Mill.] B.S.P.) communities, low organic matter quality (e.g. low %N, high C:N, high lignin, high lignin:N, low pH, low extractable inorganic N) and cold soils were associated with low rates of soil respiration, microbial turnover and gross microbial N uptake in both laboratory and field measurements. Soils from aspen (Populus tremuloides Michx.) communities had attributes of low organic-matter quality (high lignin and high lignin:N) but also attributes favorable to decomposition (high pH, high %N, high soil temperature) and exhibited much higher relative microbial activity in the field than in the laboratory, probably because warmer field conditions or other processes that occurred only in the field (e.g. root exudation) offset the effects of low organic matter quality. Field soils from birch (Betula papyrifera Marsh.) communities on warm sites also exhibited higher in situ rates of microbial activity than expected from their performance in the laboratory. Microbial activity was more important than microbial biomass in explaining community differences in soil respiration and N cycling.

Addition of labile carbon (C) and nitrogen (N) substrates to soils in the field and in the laboratory permitted microbial resource limitations to be evaluated. Microbial response to added N was greatest when labile C was abundant. Microbial demand for available soil N was greatest in soils with the highest organic C concentrations and the lowest rates of N mineralization. These observations support the conventional concept that microbial activity responds to a balanced supply of C and N. However, microbial respiration responded more strongly to sucrose (field) and cellobiose (laboratory) than to cellulose addition, indicating that the degree of defined C limitation depends on the nature of the substrate added and the ability of microbial populations to utilize it. Respiration and N immobilization responded more strongly to substrate additions than did microbial biomass, suggesting that the nature of resource limitation depends on the particular microbial parameter considered. The response of microbial respiration to added C and N also depended on the quality of native soil organic matter. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Boreal forest; Nutrient cycling; Microbial biomass; Respiration; Mineralization; Immobilization; Carbon; Nitrogen; Organic matter; 15N; Nutrient limitation of taiga communities; Soil microbial C vs. N limitation; Soil organic matter quality

1. Introduction

The productivity of any ecosystem is a function of the productive potential of component species and the degree to which environmental factors limit that potential. This concept has been extensively explored in plants, beginning with the work of Liebig in the late 19th century. Although changes in plant growth and allocation in response to changing supplies of light, water, or nutrients are widely recognized (Mooney, 1972; Chapin III, 1980; Tilman, 1985; Ingestad and Ågren, 1988; Rastetter and Shaver, 1992), the nature of environmental limitation of soil microbial activity is less clearly understood. Energy supply is often viewed as the major factor limiting soil microbial activity (Richards, 1987; Tate III, 1995) because of the heterotrophic nature of decomposition. However, most soils contain 30–100 times more dead organic carbon (C) than live microbial C (Jenkinson and Ladd, 1981). Consequently, other factors such as substrate quality, nitrogen (N) availability, physical environment (e.g. temperature and moisture), and physical protection by clays have been invoked as additional controls over microbial processing of soil organic matter (Swift et al., 1979; Richards, 1987; Tate III, 1995; Mary et al., 1996). For example, decomposition of organic matter in low-N soils might be limited by an inadequate N supply for microbial activity, leading to N immobilization (Melillo et al., 1982), reduced plant productivity, and reduced input of plant litter to soil microbes (Harte and Kinzig, 1993). Alternatively, the more recalcitrant nature

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of organic C produced by plants on low-N soils (Chapin III, 1980; Hobbie, 1992) or reduced rates of root exudation might reduce the supply of labile C substrates to microbes, resulting in reduced decomposition rates through changes in the amount of microbial biomass, its activity, or the community composition of soil microbes. Finally, the relative importance of C and N limitation may differ among microbial functional groups, e.g. bacteria and fungi (Paul and Clark, 1989), or across time scales (Norton and Firestone, 1996). The purpose of our study was to examine some of the direct and indirect ways in which substrate type and nutrient availability might influence microbial use of C and N.

In most ecosystems, N availability is controlled by several aspects of the physical and chemical environment, including availability of other nutrients (partly a function of parent material), temperature, and moisture (Jenny, 1980; Vitousek and Howarth, 1991). These interactions of N availability with the physical environment further complicate interpretations of N limitation of plant and microbial processes in natural ecosystems. The productivity of Alaskan boreal forests is limited primarily by temperature and N availability (Van Cleve and Yarie, 1986), which vary dramatically over short distances due to changes in slope and aspect (Yarie, 1983; Vierreck et al., 1986). Pools of organic N in these soils are large (Van Cleve et al., 1983), yet boreal forest productivity is often limited by low N availability. In this system, soil microbial activity is clearly a limiting step in the nutrient cycle — the “eye of the needle” through which all organic materials entering the soil must pass before nutrients become available to plants (Jenkinson, 1977). In this paper we use a combination of field and laboratory experiments to address the relative importance of C and N limitation to microbial processes in soils of differing organic matter quality. We focus on surface organic soils (i.e. forest floors) in this study because they represent the zone of greatest microbial activity in boreal forests (Van Cleve and Moore, 1978).

2. Materials and methods

2.1. Study site and sample collection

The study area was located in the Bonanza Creek Experimental Forest, a 5000 ha boreal Long-Term Ecological Research (LTER) site 30 km west of Fairbanks, Alaska (65°45'N, 148°15'W) that has been the focus of intensive ecosystem studies over the past two decades (Van Cleve et al., 1983; Vierreck et al., 1983; Van Cleve et al., 1986a). It consists of a series of forest types on a common parent material arrayed along a gradient of soil temperature and fertility related to slope and aspect. North-facing slopes have the lowest soil temperatures and support unproductive black spruce (Picea mariana [Mill.] B.S.P.) forests characterized by low rates of nutrient cycling. The warmer south-, east-, and west-facing slopes are occupied by productive aspen (Populus tremuloides Michx.), and birch (Betula papyrifera Marsh.) (Van Cleve et al., 1983; Vierreck et al., 1986). Both aspen and birch forests are replaced by white spruce (Picea glauca [Moench] Voss) forests after 80–120 years (Van Cleve et al., 1983; Van Cleve and Yarie, 1986). Soils at Bonanza Creek are derived from loess overlying Precambrian schist (Vierreck et al., 1986) and are classified as Alfic Cryorthents in the birch and aspen communities studied, and Aquic Cryorthents or Histic Pergelic Cryaquepts in the spruce communities (Vierreck et al., 1986; Dyrness et al., 1989).

We chose study sites that had the same regional climate (subarctic), parent material (schist overlain by loess), potential vegetation (Alaskan boreal forest species), and age (about 70 years) (Jenny, 1980), but which differed with respect to topography and associated community and ecosystem processes. We chose four community types (north-facing spruce, south-facing aspen, north-facing birch, and south-facing birch), allowing comparisons both within and among forest community types at mid-succession. Based on previous research (Van Cleve et al., 1983, 1991), we expected that soils from the spruce community would exhibit lowest N availability due to both low soil temperature and low organic matter quality, that aspen and south-facing birch soils would be most fertile, and that birch soils from north-facing slopes would be intermediate. Factorial fertilization experiments have shown that tree growth in birch, aspen, and black spruce communities in interior Alaska is N-limited (Van Cleve, 1973; Van Cleve and Oliver, 1982; Van Cleve et al., 1986b; Chapin, unpublished data). Thus, the communities in this study are more likely to differ in the degree of nutrient limitation than in the nature of the limiting nutrient.

We chose six replicate stands of each community type. These stands were interspersed along five ridges throughout the experimental forest, allowing us to generalize results for these forest types to the region without pseudo-replication (Hurlbert, 1984). The sample from each stand was a composite of 20–30 15-cm diameter cores of the litter layer plus organic (O1 and O2) horizons. In spruce communities, live moss on the forest floor was discarded, and soil samples were taken from the top of the dead moss layer to the bottom of the organic horizons. Live moss was distinguished from dead moss based on physical appearance; moss that was structurally intact without visible signs of decomposition was considered live, regardless of color (Skre et al., 1983; Weber and Van Cleve, 1984). Samples from each plot were combined, sieved (<0.64 cm), picked free of intact root fragments, mixed thoroughly and stored at 4°C if collected in summer or frozen (~15°C) if collected in winter. Throughout this paper, we refer to these forest floor samples as “soils”, although the organic horizon from which they were sampled is chemically and physically distinct from underlying mineral soils. We identify each soil with
reference to the community type from which it was collected (e.g. aspen soil, south-birch soil).

2.2. Soil characterization

Soils collected in May 1988 were sieved and separated into subsamples that were either air-dried for chemical analysis or used directly for analysis of microbial C and N and extractable P. Total horizon weight was calculated from the total weight of sieved soil and soil moisture of duplicate oven-dried (65°C) subsamples.

On air-dried soils we measured organic C with an induction furnace (Nelson and Sommers, 1982), total N on a selenous sulfuric acid digest followed by autoanalysis (sali-cylate acid method, Kedrowski, 1983), sulfuric acid lignin and cellulose using the procedure of Goering and Van Soest (1970), and soil pH on a soil:deionized water paste using a standard electrode technique (McLean, 1982). Sample values were corrected to an oven-dry basis by drying duplicate subsamples to constant weight at 65°C.

Microbial biomass N and C were determined using chloroform fumigation–extraction and k values of 0.54 and 0.38, respectively (Brookes et al., 1985; Vance et al., 1987). Fumigated and unfumigated soil samples were extracted with 0.5 M K2SO4 and analyzed for total extractable N (the sum of organic N, NH42, NO32 and organic C. Microbial N and C were calculated as the increase in extractable N and extractable organic C, respectively, following fumigation. Filtered extracts were analyzed for NH42 using a phenol hypochlorite assay and NO32 using the Griess–Illosevay procedure in combination with a Cd-reduction column on a modified Technicon autoanalyzer II (Whitledge et al., 1981). Total N in soils and soil extracts was determined by digesting in selenous sulfuric acid followed by autoanalysis (sali-cylate acid method, Kedrowski, 1983). Organic C in extracts was determined using dichromate digestion, as described by Vance et al. (1987). Sodium bicarbonate-extractable PO42 was determined using the method of Olsen and Sommers (1982).

To aid in the interpretation of the field respiration data described below, we measured field soil moisture, extractable inorganic N and soil temperature at 10 cm for samples collected during the field respiration study. The stand differences in soil temperature at this time were representative of measurements made at our field sites from June to August of 1988–1990 (unpublished data) and at similar sites from 1989–1997 (http://www.lter.uaf.edu).

2.3. Field respiration and mineralization experiments

To determine microbial respiratory response to C and N availability, cellulose or sucrose, with and without added N (as (NH4)2SO4), was applied to 1 m² field plots in June 1990. Each treatment was replicated twice in each of four stands per community type. Cellulose or sucrose was applied to the plots at a rate of 400 g C m⁻², and (NH4)2SO4 was surface-applied at a rate of 20 g N m⁻², resulting in a C:N ratio of addition of 20:1 for plots where both C and N were added. Sucrose and N were dissolved in a small amount of water and applied in solution, and the same amount of water applied to control and cellulose-only plots. By comparison, annual C additions in litterfall are about 100, 62, and 17 g C m⁻² for birch, aspen and spruce communities, respectively (Van Cleve et al., 1983), assuming litterfall is about 40% C. Annual litterfall N additions are about 1.9, 1.5, and 0.3 g m⁻² for these respective communities (Van Cleve et al., 1983).

Field CO2 evolution was measured using soda lime to absorb respired CO2 (Edwards, 1982). Measurements were made over 35 days (June to early July) for cellulose and over nine days for sucrose (June), using inverted, weighted plastic buckets (two in each of four stands per community type) 33 cm in diameter pushed 1 cm into the forest floor. Inside each bucket, a container holding 40 g of 6–12 mesh soda lime was placed on a wire mesh stand and collected after 24–48 h. Changes in the oven dry (100°C over night) weight of the soda lime were used in calculating respired CO2.

Field N mineralization and nitrification were measured using incubations of soil cores in gas-permeable polyethylene bags (Binkley et al., 1986). Three replicate 15.2 cm diameter soil cores encompassing litter layer and organic (O1 and O 2) horizons were collected from each stand (a total of 18 buried bags for each community type). Cores were cut in half length-wise and one-half was sieved to remove roots, extracted in 0.5 M K2SO4, and analyzed for NH4+2–N and NO32–N as described above. The other half of the soil core was placed intact in a polyethylene bag and incubated in the 0–10 cm soil layer. After 31 days, incubated cores were collected, sieved, extracted, and analyzed as above. Net N mineralization was estimated as the increase in inorganic (NH4+2+NO32) N between the two time periods.

2.4. Laboratory incubation experiments

2.4.1. Microbial activity and biomass

Microbial respiration, mineralization, and biomass were also determined in laboratory incubations, using subsamples taken (prior to air-drying) from the same soil samples used for chemical characterization of soils. Incubation experiments were carried out at 10°C (a typical soil temperature in the organic mat of deciduous stands) on soils adjusted to ca. 50% of their water holding capacity (WHC) with deionized water. Soils that had water contents higher than 50% WHC were spread on plastic sheeting and air-dried slightly and carefully with periodic mixing to bring soil moisture to ca. 50% WHC. Following the water addition or drying treatment, soils were “pre-incubated” for four to six days to allow seasonal field effects and microbial stimulation effects of soil mixing and moisture and temperature changes to diminish. Baseline respiration, mineralization, and nitrification measurements were carried out using a 21-day incubation period. Moist soil samples (5–20 g dry) were
incubated in 910 ml glass jars with sealed lids and rubber septa for analysis by gas chromatography, using a Shimadzu model gc-8a thermal conductivity chromatograph. Samples were aerated every few days. Identical samples were incubated in 250 ml polyethylene bottles covered with polyethylene film (to allow gaseous diffusion) for interim destructive sampling and extractions. Net mineralization was determined by extracting and analyzing soils for NH$_4^+$ and NO$_3^-$ at the beginning of the incubation, and after 2, 4, 10, and 21 days as described above.

2.4.2. Microbial response to C and N amendments

Responses of microbial respiration, N mineralization, and microbial biomass N to varying levels of added C substrates, with and without added N, were also determined over 60 days of laboratory incubation. Soils were collected from five south-birch and five black spruce stands in summer 1989 to compare systems contrasting in productivity and rates of nutrient cycling. Cellulose or cellobiose was used as the C amendment. Although both compounds are comprised of glucose molecules, cellulose is the most common carbohydrate constituent of plant litter and contains about 14,000 glucose units per chain, whereas cellobiose is a more labile disaccharide (Schlegel, 1986). Soils were amended with one of six concentrations of milled cellulose or cellobiose (0, 3.2, 6.5, 13, 26, and 65 mg C g$^{-1}$ dry soil) with or without addition of N (as (NH$_4$)$_2$SO$_4$), for a total of 22 treatments.

The proportion of added $^{15}$N immobilized in microbial biomass was determined using fumigation–extraction (see above). Gross (i.e. total) immobilization over the initial two days of incubation was also estimated by multiplying the fraction of $^{15}$N immobilized using fumigation–extraction by the background inorganic N soil concentration, assuming that microbial uptake of labeled and unlabeled N was proportional to their relative abundance:

$$i = \left(\frac{[^{15}Nb][^{15}Na][NH_4^+-N]e}{\beta}\right).$$

where $i$ is the gross immobilization, $^{15}$Nb the $^{15}$N immobilized in microbial biomass as measured by fumigation–extraction, $^{15}$Na the $^{15}$N added to soil, and (NH$_4^+$–N)$e$ the extractable, unlabeled soil NH$_4^+$–N concentration.

2.6. Statistical analysis

One and two-way analysis of variance tests were carried out to determine the significance of community type or treatment effects. Overall treatment and community effects and effects at individual sample dates were determined using the Bonferroni procedure, correcting significance levels for the number of comparisons made (Snedecor and Cochran, 1980).

3. Results

3.1. Soil characterization

Compared to soil from the deciduous communities, soil from black spruce communities was colder and had characteristics associated with lower organic matter quality: low %N, high C:N, high lignin, high lignin:N, low pH, and a trend toward lower extractable inorganic N (Table 1). Among the deciduous communities, aspen soils tended to have higher pH and %N (favorable for decomposition) but higher lignin concentrations (unfavorable for decomposition) compared to birch soils. Soils from the north-
south-facing birch communities were chemically similar to one another except for a slightly lower pH and trend towards higher cellulose concentrations in north-birch soils. North-birch soils also had significantly higher extracellular P concentrations and tended to be colder than south-birch soils.

3.2. Community differences in microbial processes

Field soil respiration rate differed significantly ($P < 0.05$) among communities according to the following ranking: south-birch > aspen > north-birch > spruce (Fig. 1a). There was thus a trend toward higher soil respiration for communities on south-facing slopes than those on north-facing slopes (particularly spruce) which was similar to the soil temperature trend (Table 1). Communities also differed ($P < 0.05$) in net N mineralization in the field, with spruce again showing the lowest rate (actually net immobilization; Table 2). Whereas south-birch showed the highest field respiration rate (Fig. 1a), its net N mineralization rate was intermediate between the other deciduous communities and spruce (Fig. 2, Table 2). Nitrification followed the same ranking for communities as did mineralization, with only 6–12% of the mineralized N being nitrified. Spruce soils had the largest pool size of soil

Table 1
Soil characteristics expressed on a dry weight basis (mean ± 1 SE, $n = 6$ stands). Values followed by different letters within each parameter are significantly different ($P < 0.05$)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Community</th>
<th>South-birch</th>
<th>North-birch</th>
<th>Aspen</th>
<th>Spruce</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>South-birch</td>
<td>5.8 ± 0.1 a</td>
<td>5.1 ± 0.2 a</td>
<td>6.4 ± 0.2 a</td>
<td>3.7 ± 0.0 c</td>
</tr>
<tr>
<td>C (%)</td>
<td>South-birch</td>
<td>41.4 ± 0.5 a</td>
<td>42.0 ± 0.8 a</td>
<td>40.3 ± 0.9 a</td>
<td>41.9 ± 0.6 a</td>
</tr>
<tr>
<td>N (%)</td>
<td>South-birch</td>
<td>1.50 ± 0.03 a</td>
<td>1.50 ± 0.02 a</td>
<td>1.57 ± 0.03 a</td>
<td>0.83 ± 0.01 b</td>
</tr>
<tr>
<td>C:N</td>
<td>South-birch</td>
<td>27.6 ± 0.8 a</td>
<td>28.2 ± 0.8 a</td>
<td>25.5 ± 1.3 a</td>
<td>51.4 ± 1.3 b</td>
</tr>
<tr>
<td>Lignin (%)</td>
<td>South-birch</td>
<td>30.0 ± 0.5 a</td>
<td>30.8 ± 0.6 a</td>
<td>36.3 ± 1.4 b</td>
<td>38.9 ± 0.7 b</td>
</tr>
<tr>
<td>Lignin:N</td>
<td>South-birch</td>
<td>20.0 ± 0.4 a</td>
<td>20.6 ± 0.7 a</td>
<td>22.1 ± 0.6 a</td>
<td>47.8 ± 1.2 b</td>
</tr>
<tr>
<td>Cellulose (%)</td>
<td>South-birch</td>
<td>13.2 ± 0.3 a</td>
<td>15.6 ± 0.5 a</td>
<td>16.5 ± 0.3 b</td>
<td>20.3 ± 1.0 c</td>
</tr>
<tr>
<td>Organic horizon weight (g m$^{-2}$)</td>
<td>South-birch</td>
<td>2180 ± 220 a</td>
<td>3290 ± 350 a</td>
<td>3670 ± 700 a</td>
<td>6530 ± 820 a</td>
</tr>
<tr>
<td>Field microbial pools (g m$^{-2}$)</td>
<td>Microbial C</td>
<td>27.0 ± 5.5 a</td>
<td>47.2 ± 11.3 a</td>
<td>34.9 ± 4.6 a</td>
<td>67.0 ± 11.4 b</td>
</tr>
<tr>
<td>Field microbial pools (g m$^{-2}$)</td>
<td>Microbial N</td>
<td>3.7 ± 0.7 a</td>
<td>6.0 ± 1.6 a</td>
<td>5.5 ± 0.6 a</td>
<td>5.3 ± 0.7 a</td>
</tr>
<tr>
<td>Extractable inorganic N$^+$ (mg kg$^{-1}$)</td>
<td>Lab$^b$</td>
<td>102 ± 35 a</td>
<td>102 ± 41 a</td>
<td>31.9 ± 6.2 ab</td>
<td>0.20 ± 0.23 b</td>
</tr>
<tr>
<td>Extractable inorganic N$^+$ (mg kg$^{-1}$)</td>
<td>Field$^d$</td>
<td>70.8 ± 16.7 a</td>
<td>44.1 ± 7.7 ab</td>
<td>46.3 ± 28.7 ab</td>
<td>2.2 ± 0.6 bc</td>
</tr>
<tr>
<td>Extractable P (mg kg$^{-1}$)</td>
<td>South-birch</td>
<td>48 ± 5 a</td>
<td>85 ± 5 b</td>
<td>56 ± 8 a</td>
<td>41 ± 5 a</td>
</tr>
<tr>
<td>Soil moisture (% dry wt.)</td>
<td>South-birch</td>
<td>169 ± 1 a</td>
<td>212 ± 6 b</td>
<td>200 ± 1 ab</td>
<td>202 ± 20 ab</td>
</tr>
<tr>
<td>June/July soil temp. ($^\circ$C)</td>
<td>South-birch</td>
<td>12.5 ± 0.7 ab</td>
<td>9.9 ± 0.5 a</td>
<td>13.1 ± 0.8 b</td>
<td>4.0 ± 0.6 c</td>
</tr>
</tbody>
</table>

$^a$ ($NH_4^+ - N + NO_3^- - N$)

$^b$ Measured prior to lab respiration experiments.

$^c$ Approximately 50% of water holding capacity.

$^d$ Measured prior to field respiration experiments.

Fig. 1. Soil respiration in the (a) field over 35 days ($n = 4$ stands) and (b) laboratory over 21 days ($n = 5$ stands) for south-birch, north-birch, aspen and spruce communities. Data are means ± SE.
organic matter and microbial C (Table 1) but the lowest rates of soil respiration, N mineralization, and nitrification (Fig. 1a, Table 2), indicating that community differences in microbial biomass were poor predictors of soil activity.

Laboratory incubations allowed a comparison of microbial activity in a common physical environment in the absence of plant roots and associated mycorrhizal fungi. As in the field, there were significant community effects on soil activity, but the relative rankings among communities differed from those in the field. In the laboratory, aspen soils had respiration and N mineralization rates that were just as low as those of spruce, whereas north- and south-birch soils had rates of respiration and N mineralization which were similar to one another and higher than those of aspen and spruce soils (Figs. 1b and 2; Table 3). These community differences in respiration and N mineralization were most pronounced in the first week of the incubation, suggesting that the birch soils had higher initial concentrations of labile C than did the aspen or spruce soils. This labile C pool may have been rendered available under laboratory conditions that were more optimal than those existing in the field.

The microbial biomass C per gram dry soil did not differ \((P > 0.05)\) among the four communities, although values tended to be higher in birch soils than in aspen or spruce soils (Table 4). This was a similar trend among soils as found for laboratory respiration (Fig. 1b). Microbial C comprised a similar percentage of total soil C (2.4–3.3%) across all communities. There was a significant \((P < 0.05)\) overall community effect on microbial N and microbial C/N ratio, largely due to the low concentration of microbial N in spruce soils (Table 4). Turnover times for microbial C and N in laboratory incubations tended to be longer in spruce and aspen soils than in birch soils. The extremely slow turnover of microbial N calculated for spruce soil was related to near-zero inorganic N concentrations (Table 1).

Spruce and aspen soils, which had the lowest laboratory respiration and net N mineralization rates (Table 3), showed the highest \((P < 0.05)\) proportion of added \(^{15}\)N immobilized in laboratory incubations. Over 90% of added \(^{15}\)N immobilized in microbial biomass after two days (Fig. 3a), indicating a strong microbial demand for N relative to that available in soils with low microbial activity. By contrast, south-birch and north-birch soils immobilized only 65 and 50%, respectively, of added \(^{15}\)N in microbial biomass after two days. Added N also remained immobilized for a longer period of time in spruce soils than in soils from the other communities. After 21 days incubation, twice as much of the added \(^{15}\)N remained immobilized in spruce soils compared with north-birch soils. Despite a smaller quantity of \(^{15}\)N added to spruce soil \((^{15}\)N added in proportion to background inorganic N concentrations, see Materials and Methods), the amount of \(^{15}\)N immobilized per unit microbial N (i.e. the microbial \(^{15}\)N concentration) was higher \((P < 0.05)\) in spruce soils than in the other soils (Fig. 3b), again indicating a high microbial demand for N. As expected, those soils showing the lowest proportion of added microbial \(^{15}\)N immobilized (i.e. birch soils) had the highest \((P < 0.05)\) proportion of added \(^{15}\)N in the \(K_2SO_4\) extractable (organic + inorganic) pool, even after 21 days (Fig. 3a).

Gross N immobilized as estimated by the fumigation–extraction was highest (and similar) in the two birch soils, more moderate in aspen, and very low in spruce (Table 5). This indicates that the large proportion of \(^{15}\)N immobilized in spruce soils (Fig. 3a) reflects the small quantity of inorganic N available with a slow rate of turnover (Table 4) rather than a high absolute quantity of N immobilized.

### 3.3. Carbon and nitrogen effects on soil respiration

Soil respiration in the field was strongly stimulated \((P < 0.05)\) by sucrose addition in all communities (Fig. 4). Except in spruce, the magnitude of the increase was nearly 100% and was clearly evident within one day of application, suggesting that it initially stimulated microbial activity rather than growth. There was also a strong sucrose \(\times\) N interaction \((P < 0.05)\), such that N alone had no effect on respiration but sucrose + N resulted in a near two-fold increase in respiration compared to that in the sucrose-only treatment. The sucrose \(\times\) N interaction required more time to develop than the sucrose effect, possibly suggesting an effect on microbial growth. Spruce soil respiration responded much less to sucrose and sucrose + N and was more delayed than in soils from the other communities.

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**Table 2**

Field net mineralization and net nitrification over 35 days (mean ± 1 SE, \(n = 18\) cores). Values followed by different letters within each parameter are significantly different \((P < 0.05)\).

<table>
<thead>
<tr>
<th>Community</th>
<th>Mineralization (µg N g(^{-1}) soil d(^{-1}))</th>
<th>Nitrification (µg N g(^{-1}) soil d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>South-Birch</td>
<td>1.8 ± 0.4 ab</td>
<td>0.10 ± 0.06 ab</td>
</tr>
<tr>
<td>North-Birch</td>
<td>4.1 ± 1.0 a</td>
<td>0.48 ± 0.19 a</td>
</tr>
<tr>
<td>Aspen</td>
<td>3.6 ± 1.6 a</td>
<td>0.23 ± 0.12 ab</td>
</tr>
<tr>
<td>Spruce</td>
<td>−0.06 ± 0.02 b</td>
<td>0.00 ± 0.00 b</td>
</tr>
</tbody>
</table>

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**Fig. 2.** Laboratory mineralization over 21 days \((n = 5\) stands) for south-birch, north-birch, aspen, and spruce communities. Data are means ± SE.
In contrast to the sucrose addition, cellulose did not significantly ($P < 0.05$) affect field respiration in any community (Fig. 5). Nitrogen addition stimulated ($P < 0.05$) respiration in the north-birch, aspen, and spruce soils about one month after application (Fig. 5). There was a significant ($P < 0.05$) cellulose × N interaction only in south-birch soils, where cellulose stimulated respiration only in the presence of added N. The long time lag before soil respiration responded to added N could suggest a plant-mediated response (e.g. root or mycorrhizal respiration) rather than a direct microbial response, although we have no direct evidence to substantiate this hypothesis.

In summary, field soil respiration responded primarily to labile C (sucrose) in the short-term (<9 days), but also responded to N in the longer-term (20–40 days). The respiratory response to N was induced more strongly and rapidly by sucrose than by cellulose.

Laboratory studies of C and N amendments to south-birch and spruce soils allowed a more detailed examination of C × N interactions under controlled conditions and in the absence of plant roots and associated mycorrhizae. As in the field, there was an overall significant ($P < 0.05$) effect of labile carbon substrate (cellobiose) on laboratory respiration in both south-birch and spruce soils ($P < 0.05$; Fig. 6). Individual cellobiose treatments were only significantly ($P < 0.05$) higher than the control at the three highest amendment rates in birch and at the two highest amendment rates in spruce, however. The stimulation due to cellobiose was observed at the first sample interval (two days) and disappeared within a month, presumably due to exhaustion of added substrate. Added N stimulated ($P < 0.05$) cellobiose decomposition only at the highest C addition level (65 mg g$^{-1}$) in south-birch soils and at the two highest C addition levels in spruce soils.

In contrast to cellobiose, cellulose alone stimulated ($P < 0.05$) respiration only at the two highest addition levels in south-birch soils (Fig. 7), and this stimulation was detectable only after two weeks. Nitrogen alone significantly suppressed respiration. However, N in combination with cellulose (only at the three highest levels) stimulated ($P < 0.05$) respiration and reduced the time for a respiratory response to be detected (data shown only for the two highest addition levels; Fig. 7). This resulted in a significant ($P < 0.05$) N × cellulose interaction. Thus, substrate limitation to respiration was demonstrable only at relatively high rates of cellulose and cellulose + N addition. Respiration in these treatments returned to control levels in all but the highest cellulose addition level by the end of the experiment, indicating exhaustion of added substrate. In contrast to birch, there was little detectable effect of cellulose or N addition on respiration in spruce soils (Fig. 7). Although there was a significant ($P < 0.05$) N × cellulose interaction in spruce soils, this effect was much less pronounced and more delayed than in birch soils.

### Table 3

Cumulative respiration, net mineralization, and net nitrification during 21 days laboratory incubation (mean ± 1 SE, $n = 6$ stands). Values followed by different letters within each parameter are significantly different ($P < 0.05$)

<table>
<thead>
<tr>
<th>Community</th>
<th>Respiration (mg CO$_2$ C g$^{-1}$ soil d$^{-1}$)</th>
<th>Mineralization (µg N g$^{-1}$ soil d$^{-1}$)</th>
<th>Nitrification (µg N g$^{-1}$ soil d$^{-1}$)</th>
<th>Mineralized C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>South-birch</td>
<td>0.28 ± 0.02 ab</td>
<td>1.89 ± 1.08 ab</td>
<td>0.07 ± 0.04 a</td>
<td>146</td>
</tr>
<tr>
<td>North-birch</td>
<td>0.30 ± 0.02 a</td>
<td>4.60 ± 1.06 a</td>
<td>0.08 ± 0.05 a</td>
<td>66</td>
</tr>
<tr>
<td>Aspen</td>
<td>0.19 ± 0.00 b</td>
<td>−0.49 ± 0.29 b</td>
<td>0.00 ± 0.00 a</td>
<td>^a</td>
</tr>
<tr>
<td>Spruce</td>
<td>0.17 ± 0.01 b</td>
<td>0.01 ± 0.01 b</td>
<td>0.00 ± 0.00 a</td>
<td>^a</td>
</tr>
</tbody>
</table>

^a Values not calculated due to extremely low or negative mineralization rates.

### Table 4

Microbial biomass in organic horizons (mean ± 1 SE, $n = 6$). Values followed by different letters within each parameter are significantly different ($P < 0.05$)

<table>
<thead>
<tr>
<th>Community</th>
<th>Microbial C (mg g$^{-1}$ soil)</th>
<th>Microbial N (mg g$^{-1}$ soil)</th>
<th>MC/MN</th>
<th>Microbial C (% of soil organic C)</th>
<th>Microbial turnover time</th>
</tr>
</thead>
<tbody>
<tr>
<td>-----------</td>
<td>-------------------------------</td>
<td>-------------------------------</td>
<td>-------</td>
<td>-------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>South-birch</td>
<td>12.1 ± 2.2 a</td>
<td>1.8 ± 0.3 a</td>
<td>7.1 ± 1.0 a</td>
<td>2.9 ± 0.5 a</td>
<td>Microbial C: Day 2^a</td>
</tr>
<tr>
<td>North-birch</td>
<td>13.8 ± 2.2 a</td>
<td>1.6 ± 0.3 ab</td>
<td>8.3 ± 1.5 a</td>
<td>3.3 ± 0.6 a</td>
<td>Microbial C: Day 4^b</td>
</tr>
<tr>
<td>Aspen</td>
<td>10.7 ± 1.6 a</td>
<td>1.7 ± 0.2 ab</td>
<td>6.4 ± 0.3 a</td>
<td>2.6 ± 0.4 a</td>
<td>Microbial C: Day 0–2^c</td>
</tr>
<tr>
<td>Spruce</td>
<td>10.1 ± 0.6 a</td>
<td>0.8 ± 0.04 b</td>
<td>12.4 ± 0.7 b</td>
<td>2.4 ± 0.1 a</td>
<td>Microbial N: Day 2^a</td>
</tr>
</tbody>
</table>

^a Microbial C turnover, at day 2 = microbial C/respiration C at day 2.
^b Microbial C turnover, at day 4 = microbial C/respiration C at day 4.
^c Microbial N turnover (day 0–2) = microbial N/gross N immobilized (fumigation–extraction) over days 0–2 (from Table 5).
3.4. Carbon and nitrogen effects on microbial N immobilization

The disappearance of inorganic N (added + initial) over the 60 days incubation period provides a direct estimate of net microbial N uptake. This estimate includes microbial biomass N present at the end of the experiment plus N that was immobilized and subsequently released as enzymes or as dead microbial cell fragments. Added C substrate significantly increased N immobilization ($P < 0.05$), regardless of soil type (birch or spruce), substrate type (cellobiose or cellulose), or soil N status (N added or not added) (Fig. 8; Table 6). The only exception to this result was when cellulose was added to spruce soils in the absence of added N, where no N immobilization was detected (Table 6). Soil type, C substrate type, and N addition all affected the magnitude of N immobilization in response to C addition.

The most dramatic response to C addition occurred in birch soils, where net N mineralization occurred below 13 mg C g$^{-1}$ soil (cellobiose or cellulose), and net immobilization occurred at higher levels of C addition (Fig. 8; Table 6). In the presence of added N, the proportion of inorganic N that was immobilized at the highest C addition rate was greater with cellulose (100%) than with cellobiose (90%) (Fig. 8). This may reflect the exhaustion of the less resistant cellobiose by the end of the experiment (Fig. 6). Without added N, most (89–97%) of the initial soil inorganic N was also immobilized at the highest C addition (Table 6).

The effect of C substrate on N immobilization by spruce soils differed in several respects from the patterns observed in birch soils. In contrast to birch soils, N added to spruce soils was immobilized at all C addition rates, ranging from 40% of added + initial N with no C addition to 100% of added + initial N with the highest cellobiose addition rate.
(Fig. 8). The lower sensitivity of spruce N immobilization to cellulose addition, relative to birch (Fig. 8), is consistent with the low sensitivity of microbial respiration to cellulose addition in spruce soil (Fig. 7). Without added N, calculated immobilization rates in spruce soils were close to zero due to low background soil concentrations (<1 μg N g⁻¹ soil; Table 6).

Microbial biomass N (Fig. 9) was much less sensitive to addition of C substrates than was either microbial respiration (Figs. 6 and 7) or the amount of N immobilized by microbes (measured as the change in inorganic N) over the course of the experiment (Fig. 8; Table 6). This suggests that the major effects of C substrate addition were on microbial biomass turnover and/or activity rather than on amount of microbial biomass occurring following substrate utilization. Cellobiose significantly enhanced (P < 0.05) microbial N in birch soils (with added N), but significantly decreased microbial N in spruce soils (without added N) (Fig. 9). By contrast, cellulose had no significant effect (P < 0.05) on microbial N during laboratory incubations of either soil with or without added N (Fig. 9). Both results indicate greater microbial population responses to the more labile substrate. Nitrogen addition significantly increased (P < 0.05) microbial N in spruce soils but significantly decreased (P < 0.05) microbial N in birch soils (although the effect was not significant in the cellulose experiment), suggesting a stronger N limitation or slower turnover of microbial biomass in spruce than in birch soils.

4. Discussion

Our results show two striking patterns. Firstly, the ranking field process rates among communities differed from their relative ranking in the laboratory, indicating important controls over soil activity in the field that are not adequately captured in controlled laboratory incubations. Secondly, variations among communities and among resources affected individual microbial processes differently, indicating that the concept of limitation cannot be applied simply to soil microorganisms.

4.1. Community differences

Our laboratory incubations indicate that organic matter quality and/or the associated microbial community contribute to the large differences in soil respiration, productivity and N cycling among Alaskan boreal ecosystems (Fox and Van Cleve, 1983; Flanagan and Van Cleve, 1983; Van Cleve et al., 1986; Van Cleve and Yarie, 1986). Under favorable laboratory conditions, black spruce soils exhibited low rates of soil respiration, low microbial uptake (i.e. gross immobilization), low net mineralization and nitrification, high microbial C:N ratios, high ¹⁵N retention by microbes, and slow microbial C and N turnover. Low microbial activity in spruce soils persisted even when N was added, so it cannot be entirely explained by microbial N limitation. These microbial responses are logical consequences of several indices of low organic-matter quality (high C:N, high lignin, high lignin:N), as observed previously in Alaskan black spruce ecosystems (Van Cleve et al., 1983, 1986b; Flanagan and Van Cleve, 1983) and elsewhere (Melillo et al., 1982; Berendse et al., 1987; Taylor et al., 1989), indicating that organic matter quality must have been an important...
control over microbial activity. The lack of microbial response (respiration, N immobilization, biomass N) to cellulose addition in the spruce soils shows that quantity of soil C was also not limiting. Our results show that the combined effects of low organic matter quality and low N availability in spruce soils are likely to be important in reducing microbial N uptake and increasing microbial N retention. The effective microbial sequestration of N in spruce soils likely reduces the amount of N available for plant uptake and contributes to the low rates of N uptake and cycling and low productivity observed in Alaskan black spruce forests (Van Cleve et al., 1983, 1986a).

A surprising result from the laboratory incubations was that microbial process rates in aspen soils were generally as low as those in spruce soils. Similarly, Van Cleve et al. (1986b) observed that laboratory N mineralization rates were much lower in soils from aspen communities than in soils from birch communities. Aspen stands have the highest rates of soil C and N cycling and the highest productivity of any upland ecosystem type in interior Alaska (Van Cleve and Yarie, 1986; Van Cleve et al., 1986a). A high lignin concentration was the only chemical indicator of low organic matter quality in aspen soil, suggesting that this and/or other unmeasured substrate quality parameters restrict microbial activity under favorable laboratory conditions. More importantly, the rapid N cycling and high N availability characteristic of aspen soils in the field must reflect processes that were absent or reduced under laboratory conditions. For example, root exudation, invertebrate grazing of microbes, or the activity of mycorrhizal or other fungal networks could compensate for or negate the effects of low aspen organic matter quality evident in laboratory experiments. Another striking community difference observed in laboratory incubations was the high initial respiration rates of birch soils, which presumably reflected labile C pools that were larger or more readily released under favorable laboratory conditions than those in aspen or spruce soils.

In contrast to temperate forest ecosystems where field pools of microbial biomass C and N correlate positively with primary productivity and N cycling (Myrold et al., 1989; Zak et al., 1994), we found the largest microbial C pools in soils from unproductive black spruce communities and smallest microbial C and N pools in soils from productive south-birch (Table 1). This suggests that community differences in microbial biomass pools were a consequence rather than a cause of contrasting organic matter accumulation. Similarly, microbial biomass was negatively correlated with nutrient release and plant growth in tropical forests (Singh et al., 1989). In laboratory incubations, we observed large differences in microbial activity and turnover among communities, despite similar concentrations (per g soil) of microbial biomass. This also suggests that community differences in microbial activity per unit biomass were more important than the amount of microbial biomass in determining observed patterns of C and N processing during
incubations. Large community differences in microbial activity per unit biomass probably reflect differences in:
(1) the proportion of inactive (dormant) microbial biomass;
(2) the degree of substrate limitation of microbial activity
(see resource limitation); and/or (3) the metabolic rates,
turnover, and growth efficiency of different microbial functional groups (Swift et al., 1979; Burns, 1983; Richards,
1987; Tate III, 1995).

The high concentration of microbial C (per g soil) in
boreal soils is probably a consequence of their high organic
matter content, because their microbial C per unit total
organic C is in the general range (1–3%) of that in tempe-
rate mineral soils (Jenkinson and Ladd, 1981; Martikainen
and Palojarvi, 1990; Wolters and Joergensen, 1991; Spar-
ling, 1992; Wardle, 1992). The high microbial C:N ratio that
we observed in acidic spruce soils has also been found in
other forest soils (Vance et al., 1987; Joergensen et al.,
1995; but see Martikainen and Palojarvi, 1990; Badalucco
et al., 1992) and could reflect the dominance of fungi,
including mycorrhizae, which have C:N ratios 10:1 or
greater compared with ratios of 4:1 to 6:1 for soil bacteria
(Tate III, 1995). In general, the microbial C:N ratios we
observed were within the range of those measured by fumi-
gation–extraction in other soils (Martikainen and Palojarvi,
1990; Badalucco et al., 1992; Cheng and Virginia, 1993;
Joergensen et al., 1995).

Table 6
Net N immobilization in south-birch and spruce soils amended with cellulose or cellobiose without added N over 60 days laboratory incubation (mean ± 1 SE, n = 5) (measured by change in soil inorganic N (NO$_3^-$–N + NH$_4^+$–N) over 60 days incubation; negative values depict N mineralization). Values followed by
different letters within each soil are significantly different (P < 0.05)

<table>
<thead>
<tr>
<th>C added (mg C g$^{-1}$ soil)</th>
<th>Cellobose (µg N g$^{-1}$ soil d$^{-1}$)</th>
<th>Cellulose (µg N g$^{-1}$ soil d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>South-birch $^a$</td>
<td>Spruce</td>
</tr>
<tr>
<td>0</td>
<td>$-4.6 \pm 0.6$ a</td>
<td>$-0.06 \pm 0.01$ a</td>
</tr>
<tr>
<td>3</td>
<td>$-2.6 \pm 0.7$ ab</td>
<td>$-0.06 \pm 0.03$ ab</td>
</tr>
<tr>
<td>6</td>
<td>$-0.8 \pm 0.6$ b</td>
<td>$-0.03 \pm 0.01$ ab</td>
</tr>
<tr>
<td>13</td>
<td>$2.2 \pm 0.8$ c</td>
<td>$-0.01 \pm 0.00$ ab</td>
</tr>
<tr>
<td>26</td>
<td>$3.4 \pm 0.5$ c</td>
<td>$-0.01 \pm 0.00$ ab</td>
</tr>
<tr>
<td>65</td>
<td>$3.9 \pm 0.5$ c</td>
<td>$0.00 \pm 0.00$ b</td>
</tr>
</tbody>
</table>

$^a$ Response to cellulose and cellobiose in south-birch soil were determined in separate experiments, and zero-amendment values were slightly different.
Under field conditions, rates of all microbial processes were lower in cold black spruce communities and higher in warm aspen communities than observed in our laboratory incubations, indicating the key role of soil temperature and/or correlated processes in regulating microbial activity in Alaskan boreal forests (Van Cleve et al., 1983, 1991). Root respiration, which accounts for more than half of soil respiration in Alaskan boreal forests (Ruess et al., 1996), could also contribute to differences in patterns observed between laboratory and field measurements. The high temperature sensitivity of roots compared to soil microbes (Boone et al., 1998) could explain why soil respiration is consistently highest in south-facing sites (aspen and south birch) in the field but not in laboratory incubations. Root growth may also explain the long-term (20 days) response of soil respiration to N addition in the field that did not occur in laboratory incubations. Other potentially important processes that could explain observed differences between field and laboratory results include wetting–drying and freeze–thaw cycles (Clein and Schimel, 1994; Schimel and Clein, 1996) and trophic interactions (Clarholm, 1985).

4.2. Microbial resource limitation

Our results show that a simple categorization of soil microbes as C- or N-limited ignores a diversity of microbial responses by different processes and populations. Both laboratory and field substrate-addition experiments showed that the microbial response to C limitation depended on the availability of N, the nature of the C substrate, and factors associated with soil type (e.g. organic matter quality, pH, and probable differences in microbial community composition).

Microbial respiration and N immobilization generally responded most strongly to large C additions when N was abundant and in soils characterized by high rates of N mineralization (Figs. 4–9). This finding is consistent with hypotheses based on the concept of microbial C vs. N limitation and with observations from other studies of microbial responses to labile C additions (Moore, 1981). Nitrogen addition has also generally stimulated cellulose decomposition in other studies, with the extent of stimulation dependent on substrate C:N ratio and soil N availability (Schmidt and Ruschmeyer, 1958; Rosswall, 1974; Knapp et al., 1983; Entry and Backman, 1995; Mary et al., 1996). In spruce soils, which had lower available N than birch soils (Tables 1 and 2), N immobilization and respiration required larger C additions to elicit a response than in birch soils, consistent with the generalized C/N limitation concept.

The inhibition of microbial respiration by the addition of N alone in south-birch soils in the laboratory has been observed in some boreal forest soils (Foster et al., 1980; Söderström et al., 1983) and in agricultural soils (Mary et al., 1996), but other boreal soils show no respiratory response to N addition (spruce in the current study; Moore, 1981; Salonius, 1972). Since added N stimulated respiration in the presence of an available C source, it is
unlikely that the N-induced inhibition is a simple toxic or osmotic effect. Added N may reduce C availability to microbes by condensing with soil humus (Nohrstedt et al., 1989). Nitrogen addition may also repress fungal ligninolytic activity (Keyser et al., 1978) or reduce the production of enzymes to attack N-containing organic matter (Hu and van Bruggen, 1997), but this is unlikely to directly reduce C supply.

The stimulation of field respiration by added N could have reflected a root rather than a microbial response, judging from the long time lag (>2 weeks) and lack of a stimulation of respiration in root-free laboratory incubations (our results; Flanagan and Van Cleve, 1983; Flanagan, 1986). Nitrogen addition stimulates root growth in N-limited environments (Drew and Saker, 1975) such as the forest types that we studied (Van Cleve, 1973; Van Cleve and Oliver, 1982; Van Cleve et al., 1983, 1986b), and root respiration accounted for most of the total soil respiration in black spruce forests close to our study sites (Flanagan and Van Cleve, 1977; Russel et al., 1996). Similarly, increases in root biomass and activity likely contributed to the increase in field soil respiration in response to 6 years of N and P fertilization in another interior Alaska aspen stand (Van Cleve and Moore, 1978).

The nature of the C substrate strongly influenced the respiratory response to added C, presumably due to differences in the ease of enzymatic breakdown, mobility in soils, and/or microbial use. Cellulose breakdown requires a cellulase enzyme complex whereas sucrose and cellulobiose are disaccharides, whose breakdown products can be absorbed following attack by a single enzyme (Schlegel, 1986; Paul and Clark, 1989). In addition, both cellulose and the cellulase enzyme system are relatively immobile in soils (Swift et al., 1979; Burns, 1983), so that the synthesis of cellulase may be less sensitive to substrate availability (Manning and Wood, 1983) than would enzymes specific to more mobile substrates. Alternatively, microbes that favor labile C substrates may be more C-limited or increase their respiration rate more strongly in response to substrate addition than do cellulose decomposers. It seems unlikely that the high native cellulose concentrations in these soils (Table 1) completely saturate the C demands of cellulose decomposers, as evidenced by increased N immobilization in response to even small cellulose additions in the laboratory (Fig. 8; Table 6). Our inability to detect a general microbial response to large field cellulose additions may reflect a predominance of different processes controlling field C availability (e.g. root exudation or microbial population crashes) or to the fact that substrate was added to the soil surface (to minimize soil disturbance) rather than incorporated.

Differences in the response to C and N additions among microbial processes provide insight into nutritional controls over microbial activity. Immobilization of 15N occurred without C addition (Fig. 3a) and immobilization of added N increased in response to even small additions of C (cellulose or cellulobiose; Fig. 8), indicating that microbial N utilization had a lower response threshold to C and N supply than did respiration. This could reflect: (1) microbial N storage; (2) N uptake and release of N-containing exoenzymes at substrate addition rates that failed to stimulate microbial growth; or (3) a shift to microbial populations with higher N concentrations (e.g. decreased fungi:bacteria ratio; Paul and Clark, 1989; Tate III, 1995). In spruce soils, N addition alone increased microbial N, also suggesting increased N storage and/or a decreased fungi:bacteria ratio. Potential for N storage would appear to be most likely for fungi, since they vary more widely in N concentration and C:N ratio than do bacteria (Paul and Clark, 1989). The high proportion of added N that was immobilized in spruce soils (Fig. 8), coupled with large field microbial pools, may also indicate strong microbial competitive pressure for N that is slowly mineralized in these communities. The question remains, however, as to why added N sufficient to meet microbial demand does not also increase microbial respiration rates. These findings are consistent with those from a heath tundra ecosystem, where added N and P fertilizer accumulated in soil microbial biomass without affecting microbial growth (Michelsen et al., 1999).

Two factors not directly addressed in our study are the potential nutritional role of organic N sources and the role of mycorrhizal fungi in taiga communities. Uptake of amino acids has been demonstrated for some boreal forest trees (Näsholm et al., 1998). The occurrence of organic N uptake does not negate strong evidence that inorganic N availability is an important factor limiting the productivity of taiga ecosystems, as demonstrated by field relationships among productivity, organic matter decomposition, and N mineralization, and from nutrient manipulation experiments (Van Cleve et al., 1983; Van Cleve and Yarie, 1986). This evidence suggests that any direct organic N uptake occurring on our sites is insufficient to fully meet the N requirements of plants. Ectomycorrhizal fungi, which are active in boreal forest soils, have also been shown to decompose organic matter and directly utilize organic N sources (Smith and Reed, 1997a). Ectomycorrhizae are also likely to be more dependent on simple sugars as C sources and are likely less able to utilize complex polymers such as lignin and cellulose than are other soil fungal species (Smith and Reed, 1997b). Although our field amendment studies would have included the respiratory responses of mycorrhizal fungi, we did not investigate their specific role in organic matter decomposition.

The low respiratory response to added substrates in spruce relative to birch soil could reflect any of several factors associated with spruce soils that are known reduce the rate of cellulase production or activity: (1) low availability of native substrates for microbial growth (e.g. N and labile C) and a microbial population adapted to a low substrate environment; (2) high concentrations of tannins (Flanagan and Van Cleve, 1983) which bind enzymes (Burns, 1983; Richards, 1987; Schimel et al., 1996) and...
are microbial toxins (Swift et al., 1979); (3) high lignin concentrations, which limit enzymatic access to cellulose; (4) low pH or low cation concentrations (Van Cleve and Yarie, 1986; Table 1), which reduce cellulase activity (Schmidt and Ruschmeyer, 1958; Hope and Burns, 1987; Tateno, 1988; Raubuch and Beece, 1995) and increase stability of polyphenol–protein complexes (Richards, 1987); and (5) low temperature (Fox and Van Cleve, 1983; Table 1), which limits both microbial metabolism and enzyme activity. Endocellulase activity, in particular, decreases at soil temperatures below 5±10°C in tundra ecosystems, and cellulase activity declines at cellulose:lignin ratios below 0.5 (Linkins et al., 1984). However, microbial populations in spruce soils did respond to the addition of limiting, particularly labile, substrates, indicating that no unmanipulated factor was an absolute constraint on microbial respiration. Our results, therefore, support the general conclusion of French (1988) that decomposition is seldom limited by a single factor. He proposed that enzyme activity is constrained directly by a combination of substrate (e.g. quantity and quality of C), environment, and physical access of enzyme to substrate. In addition, rates of enzyme production are influenced by availability of appropriate decomposer organisms, which may be restricted by climatic, soil, or substrate factors.

5. Conclusion

Our results show that community differences in soil fertility in the Alaskan boreal forest, caused by differences in slope, aspect, and vegetation type, have profound effects on a range of microbial processes. Field and laboratory comparisons showed that organic matter quality, as modified by differences in the physical environment and associated plant community, was an important cause of community differences in microbial activity along this complex gradient in temperature, N availability, and organic matter quality. Moreover, even when community differences in environmental factors (i.e. soil temperature and moisture) were eliminated in laboratory incubations, there was evidence that energy supply, N supply, and other unmanipulated variables associated with organic matter quality or microbial community composition were each important in controlling microbial activity. Thus, labile C and N simultaneously limited microbial activity in these soils. Although the concept of microbial C vs. N limitation provides a superficial “first-cut” explanation of differences in microbial activity along soil fertility gradients, the mechanisms by which C and N affect microbial processes depend crucially on the nature of C inputs and the effects of native organic matter on microbial activity.

Our laboratory incubations showed that, despite similar microbial biomass C concentrations in all soils, soils with lowest organic matter quality (spruce and aspen) immobilized the highest proportion of added 15N but had the lowest respiration and net mineralization, the longest microbial C and N turnover times, and the lowest gross immobilization (uptake) of added 15N. By contrast, soils with higher organic matter quality (i.e. birch) had high gross rates of 15N uptake but turned over this immobilized N rapidly due to their high rates of respiration and N mineralization. In general, north-birch soils had highest microbial activity in laboratory incubations and spruce soils the lowest. The primary difference between field and laboratory results was for aspen, which occur on warm, south-facing slopes and had higher respiration and N mineralization rates than expected from their performance in the laboratory. This suggests that favorable environmental conditions may have partially overshadowed the negative effects of low organic matter quality in this community.

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