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REGULAR ARTICLE

Soil microbial and plant community responses to single large carbon and nitrogen additions in low arctic tundra

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Abstract Plant production and community composition in many mid- and high latitude ecosystems is strongly controlled by nitrogen (N) availability. We investigated the effects of large factorial additions of labile carbon (C) (sucrose) and N (NH_4NO_3) in a single year on soil microbial and plant biomass pools over subsequent years in a widespread low arctic mesic tundra ecosystem. Soil microbes took up large amounts of N within weeks of its addition, and this accumulation was maintained over at least 2 years. Microbial biomass C was unaffected, strongly suggesting that the addition had rapidly elevated microbial N concentrations (by ~50%). Microbial biomass N and root N (per unit soil volume) decreased with depth down through the organic and mineral layers in all treatments, indicating that most of the added N was retained within the top 5 cm of the organic layer 2 years after the additions. In contrast to N, the C additions had no significant effects. Finally, plant shoot N concentrations 3 years after the additions were significantly enhanced primarily in the evergreen species which dominate this ecosystem-type, resulting in a ~50% increase in evergreen shoot N accumulation but no corresponding change in biomass. Our study demonstrates a very rapid and substantial microbial N sink capacity that is likely to be particularly important in determining N availability to plants over weekly to annual timescales in this tundra ecosystem. Furthermore, the results suggest that the moderate increases in tundra soil N supply expected due to climate warming could be largely immobilized by microbes, resulting in slower and more evergreen-dominated plant community responses than are predicted from long-term, annually repeated, high-level fertilisation studies.

Keywords Biomass · Competition · Evergreen · Graminoid · Nitrogen immobilization · Soil depth · Tundra

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Introduction

Nutrient availability to plants and soil microbes is a fundamental control on the structure and functioning of many terrestrial ecosystems (Chapin et al. 2002). Nitrogen (N) in particular seems to be the most important element limiting plant growth in arctic mesic tundra ecosystems (Shaver and Chapin 1980), although most subsequent studies have characterised responses to N and phosphorus (P) in combination (Chapin et al. 1995; Jonasson et al. 1999b).

Soil microbes are the 'eye of the needle' *through which* most inorganic N (Jenkinson 1977), and *by which* most organic N (Schimel and Bennett 2004) is mobilised from soil organic matter into the soil solution for potential plant uptake. Accordingly, the N pool within the soil microbial biomass is particularly important not just because of its size, which is equivalent to total plant N in many tundra ecosystems (Jonasson et al. 1999a), but also because N flow through this pool (i.e. microbial N turnover) is a major pathway for N supply to plants (Chapin et al. 2002). Therefore, the temporal dynamics of the microbial pool and its N storage capacity are critical to understanding tundra ecosystem functioning.

Our understanding of the relationship between nutrient availability and arctic ecosystem functioning is dominated by experiments testing responses of plant communities to repeated high-level fertiliser additions. These studies have clearly demonstrated that sustained excess soil nutrient availability increases net primary production (Jonasson et al. 1999b; Ngai and Jefferies 2004; Shaver and Chapin 1995), often results in changes in vegetation composition (Chapin et al. 1995; Press et al. 1998), and can even enhance soil organic matter decomposition (Mack et al. 2004). In sharp contrast to plants, studies of the effects of nutrient fertilisation on soil microbes in tundra ecosystems are very scarce (Treseder 2008; Wardle 2002), and therefore the role of microbes in the plant community responses outlined above is not well understood. NPK additions to a Swedish subarctic heath site increased microbial N by ~20% within a growing season (Jonasson et al. 1996), indicating strong potential for rapid microbial accumulation of added N. However, although this stimulatory N addition effect was present after 2 years of annual additions, it was not apparent after 5 years of additions except in plots where labile carbon (C) had also been added (Michelsen et al. 1999; Rinnan et al. 2007). Labile C additions to organic soils generally increase soil respiration over the course of a few weeks, but have little impact on microbial biomass C in the same time frame (Brooks et al. 2005; Ekblad and Nordgren 2002; Hartley et al. 2010; Illeris and Jonasson 1999; Lagerström et al. 2009). However, the potential interactive effect of labile C addition in 'priming' microbial N accumulation has rarely been investigated. The Swedish heath studies cited above are important in highlighting the potential influence of labile C availability on tundra microbial N accumulation, but they were focused on longer term effects, and furthermore the impacts on plants were not characterized in the early years of the additions. Finally, these microbial responses and the tundra plant community responses cited earlier were observed when nutrients were *sustained* at high levels in the soil solution over successive years through large, repeated annual additions. Therefore, they relate to conditions where competition between soil microbes and plants for these nutrients had been increasingly diminished as the studies progressed, presumably resulting in distinctive acclimation responses over time.

^{15}N isotope tracer studies in tundra and elsewhere clearly demonstrate that microbes strongly outcompete plants for inorganic and organic N over the initial hours and weeks after addition, and that microbial turnover slowly releases tracer N for plant acquisition in subsequent months and years (Buckeridge and Jefferies 2007; Grogan and Jonasson 2003; Hodge et al. 2000; Kaye and Hart 1997; Marion et al. 1982; Nordin et al. 2004; Schimel and Chapin 1996). Only the graminoid species, which together typically constitute 5–15% of total shoot biomass in mesic tundra, seem to be able to significantly increase production within a couple of months of nutrient addition (Shaver and Chapin 1980), and rapidly accumulate added tracer ^{15}N (Grogan and Jonasson 2003). The fast growing fine root systems and low allocation to structural tissue of graminoids may provide a competitive advantage over other tundra plant growth forms in rapidly responding to increases in nutrient availability (Hargreaves et al. 2009). Furthermore, the other plant growth forms may rely more on deeper soil layers for N supply, and therefore may not respond to fertiliser N until after N sinks at the surface have been saturated and excess N has been transported down through the soil column. Thus, differences in the timing of plant growth form responses to fertiliser additions can be attributed to differences in competitive ability to respond to increasing nutrients. These results, together with the N isotope studies, suggest that microbial N uptake and storage capacities may be a critical determinant of tundra plant community responses to increased nutrient availability. Specifically, the strong and rapid N uptake capacity of soil microbes suggested by the isotope studies may imply that N availability to most

plant species is not significantly enhanced by fertilisation until *after* total additions have exceeded microbial demand. However, since isotope studies involve very low-level increases in nutrient availability, actual N uptake rates and storage capacities of soil microbes in fertilised tundra and corresponding plant community responses must be characterized to evaluate the potential importance of this concept.

Here, we investigated the effects of large factorial labile C and N additions in a single year to a mesic dwarf birch hummock tundra ecosystem that extends across a large portion of the Canadian low arctic (Porsild and Cody 1980). Our preliminary objective was to determine the relative importance of labile C and N availability in limiting soil microbial growth and N accumulation during the growing season in which the nutrients were added. Our main objective was to characterize both microbial and plant community N accumulation responses in subsequent years to determine the longer term effects of the additions. The data were used to test the following hypotheses:

1) Mesic tundra soil microbes can rapidly take up large amounts of N within weeks of fertilisation, and maintain high N concentrations over subsequent years.
2) Microbial N accumulation is enhanced within weeks of labile C addition.
3) N fertilisation enhances microbial N (per unit soil volume) throughout the organic horizon and down into the mineral layer within 2 years of its addition.
4) N fertilisation in a single year stimulates vascular plant shoot N uptake after 3 years most rapidly in graminoids, moderately in deciduous species, and least in evergreen species.

Methods

Site description

This study was conducted during the growing seasons of 2004–2007 at the Tundra Ecological Research Station (TERS) at Daring Lake, Northwest Territories, Canada (64°52′N, 111°35′W). Daring Lake is located ~300 km north of Yellowknife, in the Coppermine River drainage basin. The study site is situated in a gently sloping shallow valley between outcrops of Canadian Shield bedrock and an esker that was formed toward the end of the most recent glaciation (Rampton 2000). Characteristic tundra vegetation types in this valley include dry heath, mesic dwarf birch hummock and inundated wet sedge (Nobrega and Grogan 2008).

Our study was conducted in mesic dwarf birch (*Betula glandulosa* (Michx)) hummock tundra where the vegetation is primarily hummocks and hollows consisting of *Eriophorum vaginatum* (L.), *Ledum decumbens* (Ait), *Vaccinium uliginosum* (L.), *Andromeda polifolia* (L.), *Vaccinium vitis-idaea* (L.), and *Carex* spp. (L.). Both hummocks and hollows often have a well developed moss layer of *Sphagnum* species (L.) and *Aulacomnium turgidum* (Wahlenb.), as well as lichen cover consisting of *Alectoria ochroleuca* (Hoffm.), *Cetraria nivalis* (L.), *Briocaulon divergens* (Ach.), *Cladina rangiferina* (L.) and *Masonhalea richardsonii* (Hook.). The organic soil horizon ranged from ~6–10 cm deep and is moist through most of the growing season (Nobrega and Grogan 2008). The mineral soil beneath the organic horizon had a relatively fine silt texture in the uppermost layer and thaws to ~50 cm each year (Nobrega and Grogan 2008).

Climate records from the Daring Lake Weather Station (1996–2006; Bob Reid, Indian and Northern Affairs Canada, unpublished data) indicate daily average temperatures as low as—40°C in the winter and as high as 22°C in the summer. An average of 123 days a year are above 0°C (May–September), and the mean total growing season precipitation is ~160 mm.

Experimental treatment

We located twenty-four plots (1 m^2) of similar plant species composition within a large flat patch (~2,500 m^2) of mesic dwarf birch hummock vegetation in the early summer of 2004. Each plot was centered around a single similarly sized mature dwarf birch plant (~20 cm high), and was randomly assigned one of the following treatments: C addition, N addition, C+N addition and control (n=6). On July 10th and July 28th 2004, C (250 g of sucrose (Rodgers commercial sugar, Vancouver) containing 100 g labile C) and/or N (28.98 g NH_4NO_3 fertiliser (Westgro, Calgary) containing 10 g N) were dissolved in 2 L of water and added to C, N and C+N plots

respectively. The control plots were sprinkled with 2 L of water on each date. Thus, a total of 20 g N m^{-2} (~1.6 mg N g^{-1} dry mass of organic soil) and 200 g C m^{-2} were added to the respective treatment plots.

Sampling protocol

A small sample of soil and attached vegetation was cut out from an area midway between a hummock peak and an adjacent hollow trough within each of these plots on August 18, 2004, July 7, 2005 and July 4–20, 2006. In the first 2 years, the samples were taken from the soil surface (i.e. directly below the litter layer or from the green-brown moss transition) to a depth of 5–7 cm (mean sample volume ~165 cm^3). In 2006, the soil was split into four depth layers starting from the surface as defined above: 0–2.5 cm; 2.5–5 cm; the remainder of the brown organic layer; and the first 2.5 cm of the mineral light brown/grey-coloured sand/silt layer. Aboveground plant material and fresh surface litter were removed from all samples in each year. We separated out coarse and fine roots from the soils (~15 min per sample) to estimate root biomass, and to reduce potential root contributions to microbial biomass estimates. Belowground stems were also removed and excluded from the study. The sorted soil sample for each depth layer in 2006 was homogenised and split in two: half for total soil C and N analyses; and half for biological and chemical analyses.

Aboveground plant biomass was sampled from each plot between June 27 and July 5, 2007, (just after snowmelt, approximately 5 days after birch leaf out) by cutting out a single piece of turf (~850 cm^2 × ~5 cm deep) located at least 10 cm from all edges of the plot, and away from previously sampled areas. The sampled areas in each plot were deliberately chosen to include the birch shrub, but this was not possible in some cases (n=8; scattered randomly across treatments and therefore not introducing bias) because of proximity to holes where soil had been sampled previously. In such cases, a birch shoot was removed from elsewhere within the same experimental plot so that analysis of treatment effects on species tissue N concentrations would not be affected by low sample numbers. Vegetation was sorted into the following categories: *V. vitis-idaea*, *L. decumbens*, *A. polifolia*, *R. chamaemorus*, *A. alpina*, *V. uliginosum*, *B. glandulosa* leaves ('new growth') and shoots ('old growth'), graminoid (mostly *Carex* spp.) green leaves ('new growth') and sheaths, standing dead leaves, shoot bases, and rhizomes ('old growth'); and *Sphagnum* spp. (green tissues only). These shoot categories, and roots from the depth layer study described above, were dried for at least 48 h at 65°C before weighing and grinding in a rotary mill for total C and N analysis via combustion (CNS 2000, LECO St. Joseph MI, USA). Note that vegetation was deliberately sampled early in the summer when shoot N concentrations are generally highest before seasonal growth dilution (Hargreaves et al. 2009), but that our biomass data for new shoot growth do not reflect peak values.

Biological and chemical analyses

Soil microbial biomass C and N (MBC and MBN respectively) contents were determined using a slightly modified version of the chloroform-fumigation direct-extraction (CFE) technique (Brookes et al. 1985). Briefly, 10 g fresh mass of sorted soil was extracted by adding 50 ml of 0.5 M K_2SO_4, shaking manually several times over an hour, and allowing it to sit for 1–3 h. Subsequent tests in our lab indicated no significant chemical differences between manually shaken and automatically shaken extracts. A second sub-sample (10 g) was fumigated with ethanol-free chloroform using a vacuum pump and a darkened desiccator jar for 24 h and then extracted as described above. Extracts were filtered (GF4, Fisher) and frozen prior to transport back to Queen's University for chemical analyses. Ammonium (NH_4^+-N) and nitrate (NO_3-N) in these extracts were determined colourimetrically by automated segmented flow analysis (Bran and Leubbe AAIII, Germany) using the salicylate-dichloroisocyanuric acid and cadmium reduction-sulphanilamide methods respectively (Allen 1989). Dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) in the extracts were determined using a TOC-TN analyzer (Shimadzu, Kyoto, Japan). Dissolved organic N was calculated as follows: ($DON = DTN - NH_4\text{-}N - NO_3\text{-}N$). Nutrient concentrations in all extracts were corrected for the moisture contents of the original soil samples. MBC and MBN were calculated as the differences in DOC and DTN respectively between the fumigated and non-fumigated extracts. To account for C and N in the

microbial cell wall that is not released by the chloroform we divided the microbial biomass values by 0.35 (k_C) and 0.4 (k_N) respectively (Jonasson et al. 1996). Total soil C and N were determined by combustion (CNS-2000, LECO, St. Joseph, MI, USA) on soil samples that had been dried at 60°C for 52 h and ground with a ball mill. Dry bulk density of the soil was determined from wet volume and oven dry mass of a subsample from which the roots had been removed.

Statistical analyses

The influences of the factorial C and N additions (the main factors) over the 3 years of the study ('year'—nested within the main factors) on each of the microbial and soil solution pools were investigated using separate repeated measures multivariate analyses of variance (RM MANOVAs) (von Ende 2001) with statistical software (JMP 7.1, SAS institute). All potential interactions (i.e., CxN, CxYear, NxYear, CxNxYear) were included in the analyses but only significant interactions ($P<0.05$) are reported. Data were log-transformed before analysis to meet normality tests (Shapiro-Wilks). A three-way full factorial ANOVA (C, N and year) of these data may have been more appropriate due to substantial spatial heterogeneity within each 1 m^2 plot (Buckeridge and Grogan 2008); however both analyses produced essentially the same pattern of statistically significant results.

Effects of the C and N additions on each of the microbial and solution pools at successive soil depth layers in the third year after the amendments were tested using separate RM MANOVAs ('depth layer' nested within the main factors C and N). Data for these analyses were converted from concentrations (mass per soil dry mass) to mass per unit soil volume (mg/cm^3) to account for the varying bulk density with depth, and were tested for normality and log-transformed as above.

Two-way ANOVAs were used to test for effects of factorial C and N additions on shoot biomass and N concentrations for each species, as well as on shoot N pools for each growth form, and total plot shoot N pools. Normality tests indicated that the vegetation data did not need to be transformed. As above, all potential interactions in both the soil depth and plant analyses were included in the statistical models, but only significant interactions ($P<0.05$) are reported.

Results

Effects of C and N additions on soil biogeochemistry over successive years

Microbial biomass carbon (MBC) was not significantly affected by either C or N addition within the 2004 growing season, or in subsequent years (Fig. 1). In contrast, microbial biomass nitrogen (MBN) increased by ~50% within 3 weeks of the second N addition in 2004, and remained significantly larger in 2005 and 2006 ($F_{1,20}=34.6$, $P<0.0001$; Fig. 1). Soil microbes tended to respond to elevated N availability by rapidly accumulating N rather than by increasing biomass, resulting in higher microbial N concentrations and a significantly reduced microbial C:N across all years ($F_{1,20}=53.0$, $P<0.0001$). The strong effect of the N addition in enhancing MBN was associated

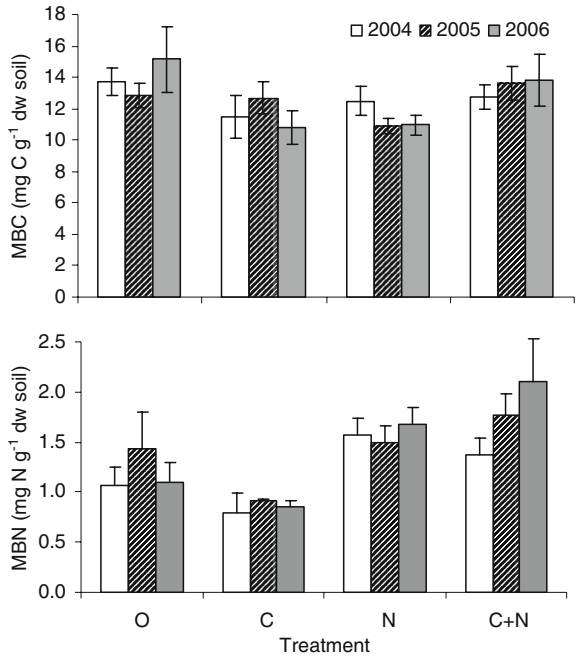

Fig. 1 Microbial biomass carbon (MBC) and nitrogen (MBN) in the factorial carbon (C) and nitrogen (N) addition plots initially and in the succeeding 2 years after the amendments ($n=6$; *error bars* represent standard errors). O indicates control plots. Data are means of the uppermost organic layer (5–7 cm deep) for each year

with a significant CxN negative interaction ($F_{1,20}$= 4.67, P<0.04) which indicated that the labile C amendment on its own tended to reduce MBN in all years (Fig. 1). Furthermore, there was a significant CxN interaction on MBC when averaged over time ($F_{1,20}$=0.05, P<0.01), indicating that microbial biomass tended to be suppressed by either factor alone, but not in combination. Overall, these CxN interactions had relatively small impacts compared to the dominant effect of N addition in stimulating rapid and prolonged microbial N accumulation (Fig. 1).

As expected, dissolved total nitrogen (DTN) in all N-amended plots was elevated within 3 weeks of the final addition in 2004 (Table 1) and remained significantly higher in 2005 and 2006, even though levels declined in all treatments over successive years (N: $F_{1,20}$=20.4, P<0.0002; Year: $F_{2,19}$=32.5, P< 0.0001). Dissolved organic nitrogen (DON) was not significantly affected by the treatments in 2004, but was significantly elevated in all N-amended plots in subsequent years (N: $F_{1,20}$=10.9, P<0.005; Year: $F_{2,19}$ =14.3, P<0.0004; Table 1). Dissolved organic carbon (DOC) pool sizes declined in successive years of the study ($F_{2,19}$=161, P<0.0001), and DTN, DON and DOC were larger and more variable in 2004 than in 2005 and 2006 (Table 1), presumably at least partly due to inter-annual variation associated with differing sample dates in each year. DTN declined rapidly over successive years reaching similar levels to DON by the end of the study (Table 1), and therefore indicating that it took 3 years for the fertiliser enhancement of the inorganic N pool to dissipate.

Effects of C and N additions on soil biogeochemistry down the soil profile

We measured several biogeochemical variables at successive soil layers in 2006 (2 years after the C and N additions) and analyzed the data per unit soil volume (mg cm^{-3}) to avoid confounding effects of increasing bulk density with depth (Organic 0–2.5 cm: 0.24 gcm^{-3} (S.E.=0.06); Organic 2.5–5.0 cm: 0.25 g cm^{-3} (S.E.= 0.05); Organic 5.0 cm-mineral: 0.39 g cm^{-3} (S.E.= 0.13); Mineral 0–2.5 cm: 1.19 g cm^{-3} (S.E.=0.22)). MBC did not significantly differ across depths but MBN per unit soil volume decreased markedly with soil depth ($F_{3,12}$=7.1, P<0.005; Fig. 2). The MBC: MBN ratio in the upper soil layers (0–5 cm) was significantly reduced by N addition ($F_{1,14}$=15.3, P< 0.002; Table 2), consistent with the N addition treatment effects on surface soil MBN over successive years reported above. Furthermore, the MBC: MBN ratio was significantly larger in the mineral soil layer than in the overlying organic horizon across all treatments ($F_{3,12}$=10.6, P<0.001; Table 2).

DTN and DON were both significantly larger in the deeper soil layers ($F_{3,18}$=19.9, P<0.0001 and $F_{3,18}$=21.3, P<0.0001 respectively; Table 2), but interactions with the amendments indicated that this effect was reduced by C ($F_{3,18}$=12.3, P<0.0002 and $F_{3,18}$=12.4, P<0.0002 respectively) and enhanced by N addition ($F_{3,18}$=4.0, P<0.02 and $F_{3,18}$=2.6, P<0.08 respectively). Consequently, as a result of these counteracting effects, DTN and DON did not vary with depth in the C+N plots (Table 2). Ammonium N

Table 1 Soil solution nitrogen and carbon contents (mg g^{-1} dry mass of soil) in the factorial carbon (C) and nitrogen (N) addition plots initially and in the succeeding 2 years after the amendments (n=6; parentheses indicate standard errors). Summary statistics report all significant effects and interactions (P<0.05)

	Year	O		C		N		C+N		Summary statistics
Dissolved total nitrogen	2004	0.19	(0.09)	0.40	(0.18)	0.69	(0.21)	0.46	(0.17)	+N↑, Year↓
	2005	0.03	(<0.01)	0.05	(<0.01)	0.30	(0.07)	0.23	(0.04)	
	2006	0.04	(0.01)	0.04	(0.01)	0.09	(0.02)	0.10	(0.03)	
Dissolved organic nitrogen	2004	0.18	(0.08)	0.39	(0.18)	0.09	(0.04)	0.25	(0.17)	+N↑, Year↓
	2005	0.03	(<0.01)	0.04	(<0.01)	0.07	(0.01)	0.08	(<0.01)	
	2006	0.03	(0.01)	0.04	(0.01)	0.07	(0.02)	0.09	(0.03)	
Dissolved organic carbon	2004	1.48	(0.18)	1.43	(0.24)	1.55	(0.05)	1.44	(0.12)	Year↓
	2005	0.48	(0.02)	0.64	(0.04)	0.60	(0.03)	0.67	(0.05)	
	2006	0.47	(0.07)	0.54	(0.04)	0.92	(0.38)	0.53	(0.07)	

Data are mean values for the uppermost organic layer (5–7 cm deep) for each year

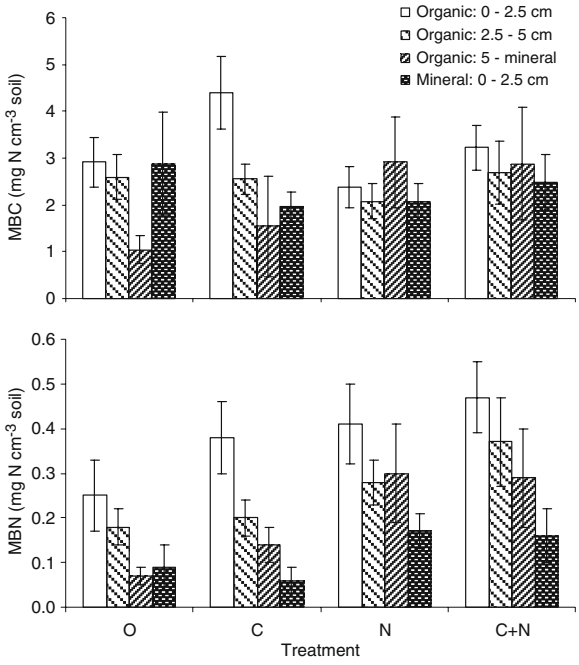

Fig. 2 Microbial biomass C and N in successive soil depth layers in 2006, 2 years after factorial additions of carbon (C) and nitrogen (N) (n=6; error bars represent standard errors). O indicates control plots

was typically 5–10% of the dissolved total N pool and did not change significantly with depth. Ammonium tended to be larger in the N-amended plots ($F_{1,20}$=3.1, P<0.09; Table 2), but not where C was also added (CxN: $F_{1,20}$=7.8, P<0.01). In association with this latter interaction, ammonium tended to be larger at depth in the N amended plots ($F_{3,18}$=10.2, P<0.0004) except where C was also added ($F_{3,18}$=13.6, P< 0.0001). Nitrate levels were generally below our analytical detection levels in all plots at all depths. Together these results indicate that N addition increased DON and ammonium contents in the lower soil depth intervals, but not where C was also added. DOC content patterns were similar, tending to be larger in the deepest soil layers in the control and N only addition plots but reduced where C was added (Depth: $F_{3,18}$=8.7, P<0.0008; CxDepth: $F_{3,18}$=3.9, P<0.03; CxN: $F_{1,20}$=6.3, P<0.02; Table 2).

Root biomass C per unit volume was generally largest close to the top surface of the organic layer (0–5 cm) and declined so severely that often there were not enough roots in the mineral layer for C and N analyses (Table 2). Even within the organic layer only, root C and N tended to decrease with depth ($F_{2,10}$=2.5, P<0.10 and $F_{2,10}$=2.4, P<0.10 respectively; Table 2). By contrast, both total soil C and N increased with depth, mainly due to relatively high pools per unit volume in the lowermost organic and mineral soil layers ($F_{3,18}$=7.0, P <0.003; $F_{3,18}$=12.4, P<0.0001; Table 2). The C and N addition treatments had no significant effects on either root C or N, or total soil C or N pools per unit soil volume.

Effects of C and N additions on vegetation 3 years after amendments

Shoot tissue N concentrations were significantly increased in several species 3 years after the addition of N, but not in response to C (Fig. 3). In particular, shoots of the two evergreen species *Ledum decumbens* and *Vaccinium vitis-idaea* (which dominate the site—Table S1), as well as *Sphagnum* mosses and *Vaccinium uliginosum* had ~45–65% higher N concentrations in N-amended plots (Fig. 3). As a result, total shoot N accumulation within the evergreen growth form was significantly increased in N-amended plots ($F_{1,20}$=5.6, P<0.03), rising from 2.08 to 3.23 g N m^{-2}. Furthermore, since evergreen species dominated the plant community (Table S1), total vascular plant shoot N accumulation was significantly increased by N addition ($F_{1,20}$=7.1, P<0.01), rising from 3.12 to 4.85 g N m^{-2}.

Discussion

Soil microbial responses to added nitrogen and carbon

Our study demonstrates that mesic tundra soil microbial communities have the capacity to rapidly accumulate large amounts of N, and maintain elevated N concentrations over subsequent years (Hypothesis 1). MBN pools were increased by ~50% within 3 weeks of the second nutrient addition without any concurrent changes in MBC. A similar rapid response has been observed in a high altitude sub-arctic fellfield, and to a lesser extent in a sub-arctic heath (Jonasson et al. 1996). Our study is novel in that we followed the impacts of the nutrient additions in 2004 over subsequent years to determine their longer term effects. The rapidly enhanced N levels within the microbial biomass were maintained over at least two successive years, while MBC was unaltered over that time. These results

Table 2 Soil biogeochemical variables (mg cm^{-3} soil) over successive depth layers in 2006, 2 years after factorial additions of carbon (C) and nitrogen (N) (n=6; parentheses indicate standard errors; b.d. indicates below analytical detection). Summary statistics report all significant effects and interactions (P<0.05)

	Depth layer (cm)	O		C		N		C+N		Summary statistics
Microbial biomass carbon: nitrogen	Organic: 0–2.5	14.83	(2.29)	12.52	(1.28)	6.14	(0.56)	7.70	(1.79)	+N↓, Depth↑
	Organic: 2.5–5	16.02	(2.17)	14.10	(1.37)	7.94	(0.78)	7.69	(0.80)	
	Organic: 5–mineral	16.46	(2.05)	15.53	(1.07)	13.33	(3.25)	9.77	(0.86)	
	Mineral: 0–2.5	27.93	(4.97)	19.66	(2.57)	15.62	(4.92)	17.98	(2.35)	
Dissolved total nitrogen	Organic: 0–2.5	0.006	(0.002)	0.016	(0.005)	0.015	(0.003)	0.021	(0.007)	Depth↑, DepthXC↓, DepthXN↑
	Organic: 2.5–5	0.009	(0.002)	0.014	(0.004)	0.021	(0.008)	0.018	(0.006)	
	Organic: 5–mineral	0.009	(0.003)	0.027	(0.008)	0.047	(0.020)	0.015	(0.005)	
	Mineral: 0–2.5	0.034	(0.014)	0.021	(0.004)	0.042	(0.011)	0.018	(0.004)	
Dissolved organic nitrogen	Organic: 0–2.5	0.006	(0.002)	0.015	(0.005)	0.011	(0.002)	0.019	(0.005)	Depth↑, DepthXC↓
	Organic: 2.5–5	0.008	(0.002)	0.013	(0.003)	0.017	(0.007)	0.016	(0.006)	
	Organic: 5–mineral	0.008	(0.002)	0.024	(0.008)	0.042	(0.018)	0.015	(0.005)	
	Mineral: 0–2.5	0.032	(0.013)	0.019	(0.003)	0.037	(0.010)	0.018	(0.004)	
NH_4^+-N	Organic: 0–2.5	b.d.		0.001	(0.001)	0.004	(0.002)	0.002	(0.002)	CXN↓, DepthXC↓, DepthXN↑
	Organic: 2.5–5	0.001	(0.001)	0.001	(0.001)	0.004	(0.001)	0.002	(0.001)	
	Organic: 5–mineral	0.002	(0.001)	0.001	(0.001)	0.006	(0.002)	b.d.		
	Mineral: 0–2.5	0.002	(0.001)	0.001	(0.001)	0.006	(0.001)	0.001	(0.001)	
Dissolved organic carbon	Organic: 0–2.5	0.097	(0.021)	0.193	(0.037)	0.121	(0.024)	0.119	(0.020)	CXN↓, DepthXC↓, DepthXN↑
	Organic: 2.5–5	0.086	(0.019)	0.152	(0.019)	0.255	(0.148)	0.111	(0.031)	
	Organic: 5–mineral	0.073	(0.013)	0.268	(0.064)	0.338	(0.130)	0.184	(0.086)	
	Mineral: 0–2.5	0.247	(0.080)	0.206	(0.028)	0.353	(0.089)	0.155	(0.023)	
Root carbon[a]	Organic: 0–2.5	7.54	(1.68)	11.08	(3.09)	9.39	(2.46)	14.64	(0.90)	
	Organic: 2.5–5	6.74	(1.45)	5.71	(1.26)	6.67	(1.52)	7.50	(1.21)	
	Organic: 5–mineral	2.84	(0.59)	4.14	(1.04)	2.59	(0.55)	2.59	(0.47)	
Root nitrogen[a]	Organic: 0–2.5	0.094	(0.02)	0.14	(0.03)	0.16	(0.04)	0.20	(0.01)	
	Organic: 2.5–5	0.098	(0.02)	0.09	(0.02)	0.11	(0.02)	0.14	(0.01)	
	Organic: 5–mineral	0.044	(0.01)	0.07	(0.02)	0.06	(0.01)	0.04	(0.01)	
Total soil carbon[b]	Organic: 0–2.5	92.3	(18.8)	174.2	(35.9)	92.4	(12.9)	106.5	(18.2)	Depth↑
	Organic: 2.5–5	111.4	(25.9)	132.8	(15.4)	92.9	(12.3)	117.1	(30.3)	
	Organic: 5–mineral	73.3	(14.8)	182.3	(47.6)	140.3	(24.8)	184.2	(74.1)	
	Mineral: 0–2.5	172.8	(40.4)	240.1	(55)	187.5	(47.6)	159.6	(27)	
Total soil nitrogen[b]	Organic: 0–2.5	2.4	(0.6)	5.4	(1.5)	2.9	(0.4)	3.0	(0.5)	Depth↑
	Organic: 2.5–5	3.5	(1.0)	5.2	(1.5)	3.5	(0.6)	4.2	(1.2)	
	Organic: 5–mineral	3.0	(0.7)	8.6	(2.6)	6.5	(1.3)	8.0	(3.6)	
	Mineral: 0–2.5	6.7	(1.5)	11.5	(2.5)	8.5	(2.4)	5.9	(1.0)	

Note that all data here are presented per unit volume to account for differences in bulk density with depth

[a] Occasional roots observed in the mineral layer were insufficient for chemical analyses

[b] Soil samples were sorted to remove most roots (see "Methods")

suggest that annual microbial turnover of the accumulated N was slow, or that N released via turnover was re-immobilised by microbes within our yearly sampling interval. By contrast, the size of the microbial biomass (MBC) was not limited by either element within 3 weeks of the factorial C and N additions, and increased C availability did not alter the strong N limitation on microbial N accumulation within that time-frame thereby refuting Hypothesis 2. We expected that effects of the C addition treatment were unlikely in subsequent years because labile C additions to tundra soils are generally respired away quickly (Brooks et al. 2005; Ekblad and Nordgren 2002; Hartley et al. 2010; Illeris and Jonasson 1999; Lagerström et al. 2009). Our results broadly support this expectation, although we did find some statistically significant but small magnitude CXN interactive effects on soil solution pools in the lower depth intervals 2 years after C addition. Accordingly, the major result from this study is that N additions in a single year had rapid and prolonged impacts of large magnitude on tundra soil microbial N pools.

The sustained microbial N sink capacity is a surprise given that many ^{15}N tracer studies suggest a mean turnover time in tundra soil microbes ranging from weeks to years (Buckeridge and Jefferies 2007; Grogan and Jonasson 2003; Sorensen et al. 2008). For example, ~50% of ^{15}N taken up by microbes in mid-summer in shrub and heath tundras was released within 4 weeks (Sorensen et al. 2008), and a similar proportion of ^{15}N taken up by microbes in early winter was released by the following July in a sub-arctic ecosystem (Grogan and Jonasson 2003).

Differences between tracer and fertiliser N retention times within the microbial biomass are likely due to the different magnitudes of N addition. Tracer amounts of isotopic N are taken up rapidly and are presumably quickly processed in the cytosol during cell metabolism under normal conditions of limiting N availability. By contrast, when microbes are exposed to large increases in N availability, the structure and composition of the soil microbial community may alter. For example, the significantly lowered microbial C:N in N-amended plots within 3 weeks of the final nutrient addition in our study may be the result of a rapid decrease in the proportion of fungi to bacteria (Paul and Clark 1996).

In addition to potential community changes, the rapid and sustained accumulation of N in microbes that we observed may also be explained by enhanced intracellular storage in pools that turn over more slowly than the cytosol. Obst and Steinbuchel (2006) determined that N-rich polypeptide-like cyanophycins may act as insoluble N storage products in many heterotrophic bacteria and cyanobacteria. Fungi contain vacuoles that are capable of storing N (primarily as N-rich basic amino acids such as arginine) as well as phosphorus and proteolytic enzymes (Griffin 1993). Furthermore, N accumulation in fungal vacuoles in response to N addition has been documented in yeast (*Saccharomyces cerevisiae)* and in *Cenococcum* mycorrhizal species (Klionsky et al. 1990; Kottke et al. 1995), suggesting that considerable N storage capacities may be present in mycorrhizal and heterotrophic soil fungi. Detailed physiological studies indicate that yeast vacuoles are involved in precise regulation of cytosolic access to, and concentration of many important metabolic elements including N, and that the vacuolar amino acid pool is large and turns over relatively slowly compared to the cytosol (Klionsky et al. 1990). Our results demonstrate that the soil microbial biomass of this mesic tundra ecosystem has a very strong physiological capacity for rapid uptake and sustained accumulation of large amounts of N from the soil solution. Future research is now required to determine the relative importance of microbial community change and microbial storage capacities in this phenomenon.

Microbial biomass N declined with increasing soil depth in all plots (Fig. 2). However, N concentrations within the microbial biomass (i.e. inverse of MBC:MBN) were significantly enhanced in the uppermost organic soil layers of all N-amended plots, but not more deeply (Table 2). Had there been downward movement of N that was accumulated by microbes at lower depths, we would have expected: a) a significant interaction between depth and N addition on MBC:MBN; and b) a decrease in MBN in the uppermost soil layer of the N addition plots over the years following fertilisation. In contrast, the results indicate that most of the accumulated fertiliser N was retained in the upper soil layers even 2 years after the addition, refuting Hypothesis 3. Since root biomass and N pools also significantly declined with depth, these results together strongly suggest that biogeochemical N cycling was most active close to the top soil surface (0–5 cm of the organic layer) in all plots.

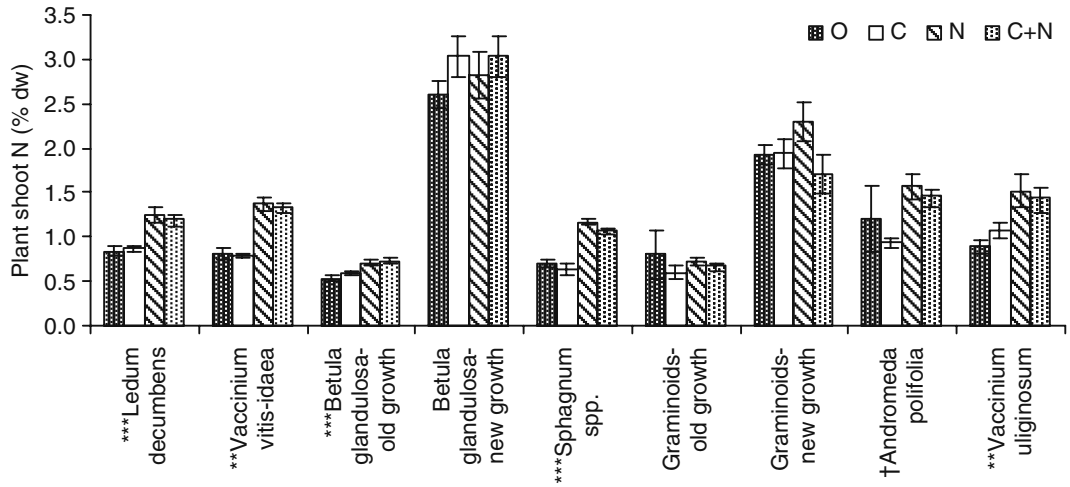

Fig. 3 Plant shoot N concentrations 3 years after the C and N additions (n=4–6; *error bars* represent standard errors). O indicates control plots. Species are listed in order of decreasing mean shoot biomass across all plots (Table S1). Statistical analyses of the effects of C and N additions using 2-way factorial ANOVAS indicated several significant N addition effects only (P values <0.001 are indicated by ***; <0.01 by **, and <0.06 by †)

Plant shoot responses to added nitrogen and carbon

Our vegetation results suggest that tundra plant communities may respond differently to a single pulse addition than to annually repeated high-level fertiliser additions. Evergreens were the principal growth form that responded to our N amendments of 3 years earlier, refuting Hypothesis 4. Shoot N concentrations of the principal evergreen species were enhanced by ~50%, resulting in significantly enhanced evergreen shoot N per m^{-2} but no significant increase in evergreen shoot biomass. By contrast, there were no significant effects on new or old shoot N concentrations in the graminoids, and a relatively small increase in N concentration within the old growth shoot tissues of *Betula glandulosa*—the principal deciduous shrub.

Shoot N pools were elevated in *all* plant growth forms within 3 years of repeated high-level fertilisation (10 g N m^{-2} y^{-1}) in similar vegetation in Alaska (Chapin et al. 1995). Repeated annual fertilisation typically results in significant corresponding shoot biomass increases over the same time frame (3–5 years) for the graminoids and deciduous shrubs, but not for the evergreens (Chapin et al. 1995; Michelsen et al. 1999; Press et al. 1998). The graminoids in particular seem to be able to significantly increase production within a couple of months of nutrient addition (Shaver and Chapin 1980), and rapidly accumulate added tracer ^{15}N (Grogan and Jonasson 2003). Very high-level fertiliser additions (25 g N m^{-2}) enhanced plant shoot N concentrations in most tundra species within a single growing season, while moderate levels (5 g N m^{-2}) had no effect over the same period (Shaver and Chapin 1980). One interpretation of our results in the context of the above fertilisation studies is that the graminoids are the first plant growth form to respond to whatever excess N is present *after* soil microbial N uptake capacity has been saturated. We found that

Table 3 The ecosystem nitrogen budgets (g N m^{-2}) of control (O) and N addition plots (n=6; parentheses indicate standard errors)

	O	N	Mean N increase
Microbial N[a]	17.6 (3.7)	31.3 (5.0)	13.7*
Dissolved total N[a]	1.3 (0.3)	3.8 (1.2)	2.6*
Plant shoot N[b]	3.4 (0.3)	5.6 (1.0)	2.1*
Total measured N	22.3	40.7	18.4[c]

* Statistically significant N increases in each variable due to N addition (P<0.05, one-tailed t tests)

[a] Microbial and dissolved total N pools were each summed across all sampled depths (mid-summer harvest 2006)

[b] Plant shoot N is summed across all species (early summer harvest 2007)

[c] Total fertiliser N addition was 20 g N m^{-2}

microbial N uptake was rapid and that the sink capacity may have matched or exceeded the total addition of 20 g N m^{-2} (see below), suggesting that immediate fertilizer N availability to plants would have declined very quickly. Although graminoids may be able to respond rapidly to high soil solution N concentrations from fertiliser additions, they may not be able to compete as effectively with other plant growth forms for N supplied via microbial N turnover. By contrast, the evergreen species with their characteristic ericoid mycorrhizae may have a particular advantage in such competition because the associated hyphae have proteolytic capacities and are more efficient than roots at taking up N per unit of C invested (Read 1991). Fungi dominate the soil microbial community biomass at our site (Buckeridge and Grogan 2008), and at other dwarf shrub tundra sites (Eskelinen et al. 2009).

Some extrapolations on the overall ecosystem N budget following N addition

Calculation of an ecosystem N budget in our study suggests that the enhanced plant N accumulation we observed was largely due to slow release of accumulated N from the microbial biomass, rather than to N fertilisation in excess of microbial demand. Rapid plant N uptake prior to the first sampling in 2004 could have occurred, but even graminoids are unlikely to have increased their N concentrations by >50% within a few weeks of added N fertiliser (Hargreaves et al. 2009), suggesting that the absolute amount they could have acquired at our site would be small (mean new growth×%N=~0.13 g N m^{-2}). In any event, assuming the K_N microbial biomass N extractability factor (see "Methods") is accurate, our budgets can account for most of the added N both initially, and up to 3 years after its addition (Table 3). In other words, the net increase in measured ecosystem N pools 2–3 years after the N addition matches the fertiliser N input. Most of the added N was immediately accumulated and retained in the microbial biomass through the soil column (~75%) with the remainder present in the soil solution (13%) and in plant shoots (11%). Since some N losses via leaching, herbivory and denitrification from these N addition plots are likely, we assume that either the N addition enhanced N availability by stimulating soil organic matter decomposition as has been observed elsewhere in deep tundra soil horizons (Mack et al. 2004), or that these losses were small in magnitude relative to the MBN pool size.

The potential significance of strong microbial N accumulation capacity in tundra ecosystems

Our study may be important to understanding tundra ecosystem responses to climate change. The magnitude of N uptake that we observed within a few weeks of its addition is an order of magnitude larger than typical rates of plant annual N uptake (1–2 g N m^{-2}) (Jonasson et al. 1999a; Shaver and Chapin 1991). Furthermore, the N addition levels used in most annually repeated fertilisation studies (Chapin et al. 1995; Michelsen et al. 1999; Press et al. 1998) are very high (typically 10 g N m^{-2} y^{-1}) compared to *in situ* rates of inorganic N supply into the soil solution (0.1–0.5 g N m^{-2} y^{-1} as determined with the buried bag net N mineralisation technique) for a variety of tundra vegetation types in Alaska (Giblin et al. 1991), Daring Lake in Canada (Nobrega, pers. comm.) and several heath tundra warming treatments in sub-arctic Sweden (Schmidt et al. 1999). Although some calculations suggest that optimal temperature and moisture conditions might produce levels comparable to the experimental fertiliser inputs in a particular year (Mack et al. 2004), it seems unlikely that such high rates of inorganic (or organic) N supply would be realistically sustained over decadal and longer time scales. Therefore, there remain many fundamental questions concerning the relationship between increasing nutrient availability due to climate warming and tundra ecosystem functioning. Our results are based on microbial and plant responses to a single large pulse N addition, and may not be indicative of the impacts of gradual increases in nutrient availability. Nevertheless, they clearly demonstrate strong soil microbial capacity for uptake and sustained accumulation of large amounts of N in a widespread tundra ecosystem type. Accordingly, this study suggests that the moderate increases in tundra soil N supply expected due to climate warming could be largely immobilized by microbes, resulting in slower and more evergreen-dominated plant community responses than are predicted from long-term, annually repeated, high-level fertiliser addition studies.

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