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Nitrogen Uptake During Fall, Winter and Spring Differs Among Plant Functional Groups in a Subarctic Heath Ecosystem

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Abstract

Nitrogen (N) is a critical resource for plant growth in tundra ecosystems, and species differences in the timing of N uptake may be an important feature regulating community composition and ecosystem productivity. We added ^{15}N-labelled glycine to a subarctic heath tundra dominated by dwarf shrubs, mosses and graminoids in fall, and investigated its partitioning among ecosystem components at several time points (October, November, April, May, June) through to the following spring/early summer. Soil microbes had acquired 65 ± 7% of the ^{15}N tracer by October, but this pool decreased through winter to 37 ± 7% by April indicating significant microbial N turnover prior to spring thaw. Only the evergreen dwarf shrubs showed active ^{15}N acquisition before early May indicating that they had the highest potential of all functional groups for acquiring nutrients that became available in early spring. The faster-growing deciduous shrubs did not resume ^{15}N acquisition until after early May indicating that they relied more on nitrogen made available later during the spring/early summer. The graminoids and mosses had no significant increases in ^{15}N tracer recovery or tissue ^{15}N tracer concentrations after the first harvest in October. However, the graminoids had the highest root ^{15}N tracer concentrations of all functional groups in October indicating that they primarily relied on N made available during summer and fall. Our results suggest a temporal differentiation among plant functional groups in the post-winter resumption of N uptake with evergreen dwarf shrubs having the highest potential for early N uptake, followed by deciduous dwarf shrubs and graminoids.

Key words: ^{15}N isotope labelling; glycine; cold-season plant nitrogen uptake; winter; temporal nitrogen uptake pattern; microbial biomass.

Introduction

High-latitude ecosystems are characterized by long winters when soils are frozen for a prolonged period of time. Although biological processes slow down during winter, these ecosystems are often far from dormant. Many studies have shown that soil

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microbial activity continues at temperatures well below zero (Clein and Schimel 1995; Zimov and others 1996; Brooks and others 1996, 1998; Hobbie and Chapin 1996; Oechel and others 1997; Fahnestock and others 1998; Jones and others 1999; Larsen and others 2007b) often resulting in peak soil microbial biomass in early spring before the onset of snowmelt and soil thaw, followed by a significant dieback during the snowmelt period (Lipson and others 1999; Grogan and Jonasson 2003; Schmidt and Lipson 2004; Edwards and others 2006; Larsen and others 2007a; Buckeridge and Grogan 2010).

The microbial dieback during snowmelt has been shown to lead to immediate or slightly delayed increased soil levels of organic and/or inorganic nitrogen (Lipson and others 1999; Schmidt and Lipson 2004; Edwards and others 2006; Buckeridge and Grogan 2010), although this is not always the case (Brooks and others 1998). As plant growth during the short summer in high-latitude ecosystems is most often limited by the availability of nitrogen (Shaver and Chapin 1980; Vitousek and Howarth 1991), it would obviously be favorable to plants to be able to take up the nutrients being released during the microbial dieback in early spring. Non-growing season uptake of nitrogen by plants is also supported by the large number of studies showing that net nitrogen mineralization during the growing season is often less than the amount of nitrogen taken up by plants each year (Hart and Gunther 1989; Giblin and others 1991; Jonasson and others 1993; Fisk and Schmidt 1995; Schmidt and others 1999, 2002; Kaiser and others 2005). In contrast, considerable net mineralization of nitrogen takes place within the winter and spring thaw periods (Hobbie and Chapin 1996; Grogan and Jonasson 2003; Schimel and others 2004; Kielland and others 2006).

A limited number of investigations of plant nutrient uptake have focused on the cold-season uptake, and their results are not consistent. For example, cold-season nitrogen uptake occurred only during early fall and after late winter in the understory of a subarctic birch forest ecosystem (Grogan and Jonasson 2003), whereas plant nitrogen uptake was high during spring snowmelt in a wet alpine meadow (Jaeger and others 1999; Bilbrough and others 2000), but very low during snowmelt in an arctic tussock tundra ecosystem (Bilbrough and others 2000). In two high-arctic tundra types, one dominated by bryophytes and *Salix polaris* and one by bryophytes, lichens and graminoids, Tye and others (2005) found that *Salix polaris* and lichens/graminoids, respectively, had the largest N uptake potential compared to other functional groups immediately after snowmelt. Recently, Edwards and Jefferies (2010) found substantial N uptake by *Carex aquatilis* in soil cores excavated during spring thaw from a low-arctic wet meadow. The contrasting results of these studies imply that we still have only fragmented knowledge of the magnitude of tundra plant nitrogen uptake capacity outside the growing season. Secondly, more studies are needed to further clarify whether intrinsic differences in cold-season nitrogen uptake among species and/or plant functional groups exist.

In the present study, we investigated the timing of nitrogen uptake by different functional groups of plants in a subarctic alpine wet heathland with focus on the cold season using a single ^{15}N addition approach. We added ^{15}N-labelled amino acid (glycine) as a stable isotopic tracer in early fall and quantified ^{15}N tracer uptake by evergreen shrubs, deciduous shrubs, graminoids and mosses at consecutive harvests in the following late fall, winter, spring and early summer. As niche differentiation has been shown both with respect to preferred N form as well as to timing of uptake during the growing season (McKane and others 2002), we expected that the magnitude of N uptake would vary between plant functional groups due to growth form differences in life-strategies and temporal patterns of photosynthetic capacities and N demands. Based on an assumed higher photosynthetic capacity of evergreen dwarf shrubs and mosses during the cold season and in particular during early spring (Oechel and Sveinbjörnsson 1978; Starr and Oberbauer 2003; Larsen and others 2007b), we expected that these growth forms would have a higher N uptake potential during the cold season than deciduous dwarf shrubs and graminoids.

Materials and Methods

Site Description and Experimental Design

The experimental site is situated near Abisko Scientific Research Station, Northern Sweden (68°21′N, 18°49′E), 380 m above sea level. Climate records (1970–2000) show a mean annual temperature of −0.5°C (−10.2°C in January and 12.6°C in July), mean annual precipitation of 315 mm, and snowcover usually from early October to mid May. For this site we thus define the cold season to be the period from 1 October to 15 May. The highly organic soil horizon overlying

bedrock contains 87.4 ± 6.8% SOM (soil organic matter) and a distinct mineral soil layer is absent. The soil has a depth of 5–20 cm and a pH of 5.8 (Konestabo and others 2007). Permafrost is patchy in the region and not present at the site. The vegetation at this wet dwarf shrub heath is dominated by mosses, evergreen and deciduous dwarf shrubs, and graminoids. Dominant vascular plants are *Empetrum nigrum* ssp. *hermaphroditum*, *Vaccinium uliginosum* ssp. *alpinum*, *Andromeda polifolia* ssp. *polifolia*, *Arctostaphylos alpina*, *Rhododendron lapponicum*, *Betula nana*, *Equisetum arvense*, *Carex vaginata*, *Carex parallela* and *Carex rupestris*.

The experimental area consisted of twelve 1 m × 1 m plots situated within an area of approximately 50 m × 50 m. One year before the present study, half of the plots had been exposed to more freeze–thaw cycles in the soil surface during spring and fall for 3 years in an experiment using small plastic greenhouses to prevent snow accumulation for about 3 weeks in each of the shoulder seasons during fall and spring (for further details refer to Konestabo and others 2007). Effects of this pre-treatment on the ^{15}N tracer content of all plant, soil and microbial fractions in the present study were tested by two-way ANOVA with sampling month and treatment as main effects and their interaction (analysis not shown). Because there were no significant treatment effects for any of the variables, we assumed that there was no effect of the pre-treatment and therefore pooled the data from all 12 plots in our results shown here.

Soil temperatures at 3-cm depth were logged at 15-min intervals in six plots (Gemini Data Loggers, Chichester, England) throughout the study and air temperatures (at 2-m height) were recorded every 2 min at a nearby weather station at Abisko Scientific Research Station. Because the total organic and inorganic dissolved nitrogen (TDN) in the soil is dominated by organic N (overall mean ratio of organic to inorganic dissolved N in the soil over the study period was approximately 44—see Figure 3B, C), we chose to add labelled N in the form of single-labelled amino acid glycine, which is a common amino acid in subarctic heath soils (Sorensen and others 2008). Field addition of labelled N into the soil is hindered for a long period during winter when the soil is frozen. Labelling of all subplots used in the experiment was performed just prior to the onset of winter soil frost, when field addition of labelled N into the soil was still possible in unfrozen soil. Consequently, five separate 20 cm × 20 cm subplots within each plot were labelled on 22 August 2003. Labelling was achieved by gently pressing a 20 cm × 20 cm perspex plate with metal pegs in each corner down on top of each subplot until the plate was 2–3 cm above the soil surface. We mixed a label solution of 20.64 mg N l^{-1} of single-labelled glycine (99% ^{15}N) and used a syringe to add 10 ml of label through each of 25 holes evenly distributed on the plate, thus adding a total of 250 ml per subplot equal to 129 mg glycine-^{15}N m^{-2}. The syringe needle was inserted down about 10 cm into the soil, and then the solution was sprayed out while drawing the syringe up to the top of the soil so as to dispense the label as evenly as possible.

Field Sampling and Laboratory Analyses

One randomly selected labelled subplot within each plot was excavated to a depth of 10 cm on each of five harvest campaigns (4 October 2003, 12 November 2003, 9 April 2004, 11 May 2004 and 21 June 2004; 60 subplots in total). The experiment thus represents a progression in time after labelling from 43 to 304 days. In May, six additional non-labelled cores were excavated for determination of background levels of ^{15}N. Excavated cores were brought to the laboratory and stored at 5°C.

Within 48 h after collection, the plants were gently pulled out of the soil cores with as many roots attached as possible. Plants were sorted into seven fractions: mosses and lichens; and shoot and roots of evergreen shrubs, deciduous shrubs and herbs. Mosses constituted more than 95% of the moss/lichen fraction, and therefore we hereafter refer to this fraction as *mosses*. Similarly, *Carex* and grass species constituted more than 90% of the herb fraction and we refer to this pool as *graminoids*. The roots were washed in 0.5 mM $CaCl_2$ to remove loosely adhered label (Persson and others 2003), and all plant parts were dried until constant mass at 70°C. The dried samples were crushed with a mill or a mortar and kept in darkness at room temperature until further analysis.

A soil subsample of about 100 cm^3 was taken from the excavated plant/soil cores after the removal of aboveground biomass and adhering roots. The exact volume of the subsample was measured and used for calculation of soil bulk density. As many remaining roots as possible were then hand-picked from the soil subsample within a standardized time of 20 min per sample. Total root biomass was determined as the sum of attached roots from all plant groups plus the remaining roots picked out from the soil subsamples.

After removal of the roots from the soil samples, about 30 g of soil per sample was freeze-dried for determination of soil water content, whereas

2 × 10 g were immediately extracted in 50 ml of demineralized H_2O and 50 ml of 0.5 M K_2SO_4, respectively, followed by filtration through a Whatman GF-D filter. Another 10 g was chloroform-fumigated for 24 h (Brookes and others 1985) before extraction in 50 ml of demineralized H_2O to release microbial nutrients. The extracts were stored frozen at −25°C prior to analysis. The water extracts were necessary to avoid large amounts of crystallized salts when extracts were freeze-dried prior to ^{15}N analysis. The extraction in demineralized H_2O compared to 0.5 M K_2SO_4 may underestimate N as well as ^{15}N tracer recovery in microbial biomass nitrogen (MBN) and TDN although Clemmensen and others (2008) found only little differences in extractability between the two methods. Still, we refrain from drawing conclusions with respect to the ratio of MBN to soil immobilized N and ^{15}N tracer recovery. More importantly, the method is consistent throughout the experimental period and relative changes over time in all pools should be similar with both extraction methods.

The 0.5 M K_2SO_4 extracts were used for determination of DOC (dissolved soil organic C) on a Shimadzu TOC-5000A total organic C analyzer and for determination of NH_4^+-N with the indophenol method (Allen 1989). TDN in the 0.5 M K_2SO_4 extracts was determined by persulphate oxidation (Zhou and others 2007), followed by the cadmium reduction method (Allen 1989). Because NO_3^--N is poorly extracted by K_2SO_4 it was determined on the pure H_2O extracts with the cadmium reduction method (Allen 1989). Dissolved organic nitrogen (DON) in the 0.5 M K_2SO_4 extracts was then calculated as TDN minus inorganic N pools.

Mass Spectrometry

Total carbon (C), total nitrogen (N) and the $^{15}N/^{14}N$ ratio in the plant and soil fractions from each harvest were analyzed on 5–10 mg of sample with a CN elemental analyzer (Eurovector, Milan, Italy) coupled to an Isoprime isotope ratio mass spectrometer (IRMS, Micromass-GV Instruments, Manchester, UK) using continuous flow. All samples were analyzed with reference gas calibrated against international standards IAEA C5, CH6, CH7, N1, N2 and USGS 25, 26, 32, and corrected for drift using internal standards of calibrated leaf material. The standard deviation of the $\delta^{15}N$ measurements was ±0.2‰.

Total C and N in the soil microbial biomass and the microbial $^{15}N/^{14}N$ isotope ratio were determined from the demineralized H_2O soil extracts. A fraction of each extract was oxidized with the persulphate method (Zhou and others 2007) and analyzed for TDN with the cadmium reduction method (Allen 1989). Knowing the N concentration, 3–20 ml of un-oxidized samples, containing about 100 µg N, was freeze-dried in 20 ml plastic vials together with a 12.5 mm^2 quartz fiber filter disc (Millipore, Billerica, USA). After drying, the filters were used to wipe any adhered dry matter off the sides and bottom of the vials. An additional quartz filter was wetted with 10-µl demineralized H_2O, and the vial was wiped again to recover as much dried material as possible. The two filters were subsequently dried in a desiccator at room temperature and analyzed as described above. TDN was determined as the N content from IRMS of the non-fumigated sample. The microbial N (MBN) contents were calculated as the difference between N content from IRMS of fumigated and non-fumigated samples. As Clemmensen and others (2008) found only small differences between microbial biomasses based on salt and water extracts, respectively, we used an extractability factor of 0.4 (Jonasson and others 1996; Schmidt and others 2002). Soil immobilized ^{15}N content was calculated as the ^{15}N content in dried soil samples minus the ^{15}N content of MBN and TDN. For aboveground plant biomass and attached root fractions we calculated ^{15}N tracer recovery (% of added ^{15}N) as:

$$\frac{(^{15}N_l - ^{15}N_b) \times \text{Biomass} \times 100}{^{15}N_a} \quad (1)$$

where $^{15}N_l$ and $^{15}N_b$ are the ^{15}N contents (g ^{15}N g^{-1} sample) in labelled samples and background, non-labelled samples, respectively. Biomass is the harvested biomass (g m^{-2}), $^{15}N_a$ is the added amount of ^{15}N tracer (g m^{-2}). For belowground fractions (MBN, TDN, soil immobilized N and total roots), we calculated ^{15}N tracer recovery as:

$$\frac{(^{15}N_l - ^{15}N_b) \times \text{BD} \times \text{Vol} \times 100}{^{15}N_a} \quad (2)$$

where BD is the bulk density (g cm^{-3}) and Vol is the soil volume down to 10 cm depth (cm^3 m^{-2}). Because biomass varies in space and time we also calculated the ^{15}N tracer concentrations relative to total N content of each pool (mg ^{15}N tracer g^{-1} N):

$$\frac{(^{15}N_l - ^{15}N_b) \times 1000}{N_{l.frac}} \quad (3)$$

where $N_{l.frac}$ is the fraction of N content to total biomass in the labelled samples (g N g^{-1} biomass). For each N pool, background levels of ^{15}N content were calculated as the mean ^{15}N content of the

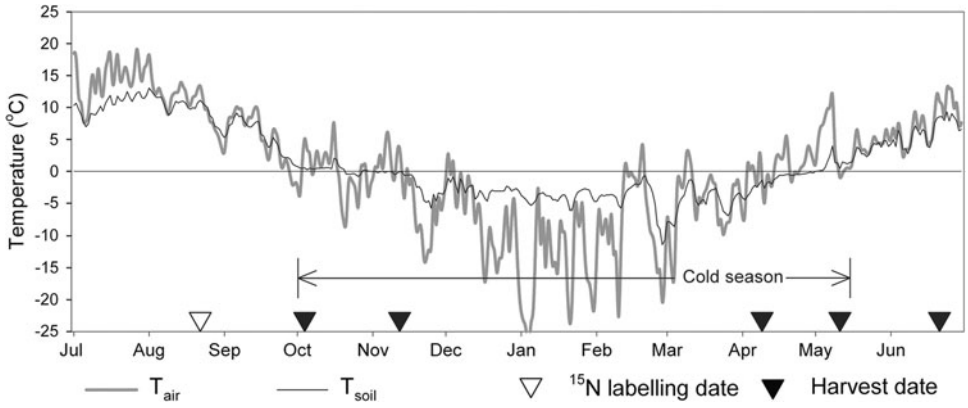

Figure 1. Seasonal progression of soil and air temperatures. T_{air} is the temperature 2 m above the ground at the nearby weather station and T_{soil} is the temperature 3 cm below the soil surface at the study site. The cold season (1 October to 15 May) is indicated as well as the times of addition of ^{15}N-labelled glycine and harvests. Note that all subplots were labelled on the same day and thus represent a progression in incubation time from 43 to 304 days.

non-labelled samples excavated in May. This procedure significantly reduced the number of background samples needed and was based on previous work indicating that natural variation in ^{15}N content was very small compared to the variation in labelled material. All data are presented relative to the amount initially added according to equations (2) and (3) (that is, ^{15}N tracer recovery), as well as relative to the N content of each pool according to equation (3) (^{15}N tracer concentration).

Statistical Analysis

The plant biomass and ^{15}N pool data were tested for effects of sampling month for each individual fraction, by one-way ANOVA followed by Tukey's test to localize the differences using $\alpha = 0.05$. Assumptions of homoscedasticity in ANOVA were tested with Levene's test, and data were log-transformed when necessary. Before running the ANOVA for ^{15}N pool differences over time, Wilcoxon's non-parametric test was used to test if δ^{15}N-values in samples from labelled plots were significantly higher than the background level in un-labelled samples.

Results

Seasonal Temperature Conditions

Mean diel soil temperature at this subarctic site was close to 0°C from the beginning of October to mid-November, and then rapidly dropped to around −5°C where it remained until mid-February (Figure 1). A warm weather front with several days of above-zero air temperatures in mid- to late-February apparently reduced the effectiveness of the insulating snow cover, resulting in considerable soil cooling and higher variability in soil temperatures thereafter. Soil temperature reached 0°C on 1 May, and then increased during the following days, indicating that snowmelt was completed.

Seasonal Biomass Carbon and Nitrogen Pools

The aboveground biomass C of both evergreen and deciduous shrubs did not change significantly over the study period, whereas aboveground graminoid biomass C was lower in November and May compared to October (Figure 2A). Aboveground biomass N showed a similar pattern, but also included a significant increase in biomass N of deciduous shoots from May to June (Figure 2B). Moss biomass C did not change over the study period (Figure 2A) whereas moss biomass N was lower in April than in October (Figure 2B). Belowground, the root biomass C and N of both evergreen and deciduous shrubs was significantly lower in the April harvest only, most likely due to difficulties in sampling the frozen hard soil at this time leading to a lower soil sample volume as well as lower biomass recovery (Figure 2A, B). Microbial biomass carbon (MBC) increased by about 55% from November to April and then decreased back to its original level by the May harvest (Figure 3A). In contrast, MBN was stable over the entire study. As for MBC, dissolved organic carbon (DOC) peaked in April and decreased significantly by May, whereas DON did not change significantly over the study period (Figure 3B). The NH_4^+ pool fluctuated, reaching the highest levels in November and lowest levels in April, whereas NO_3^- showed the

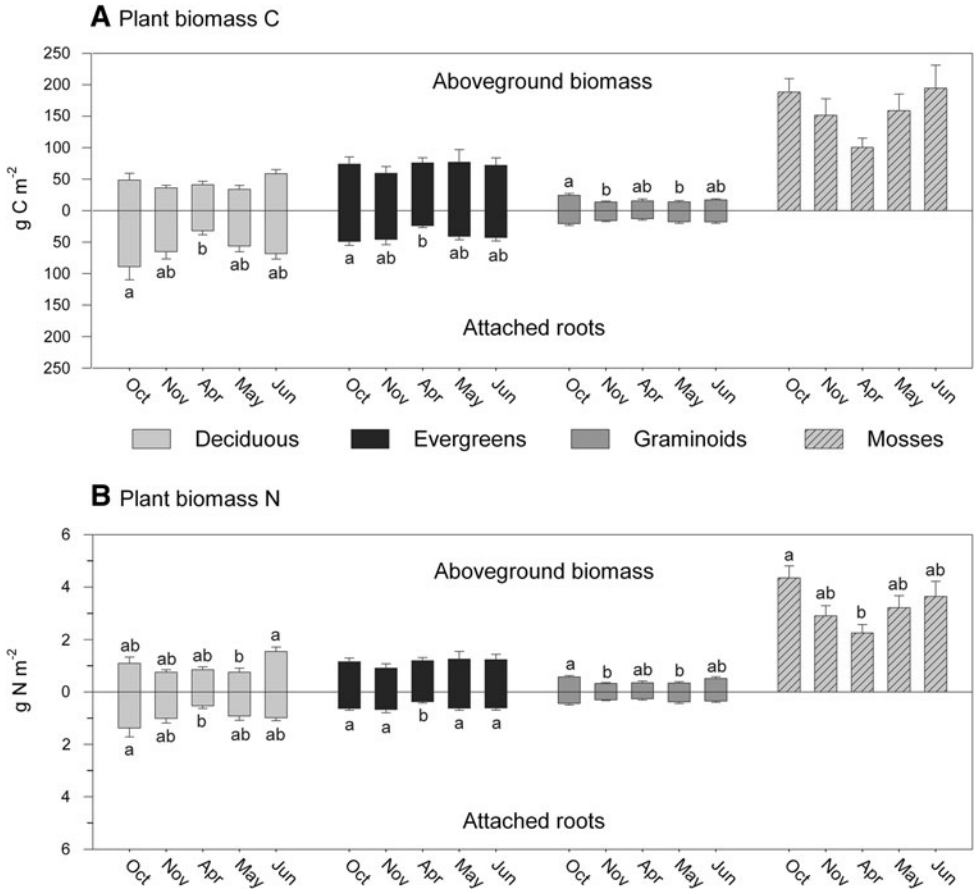

Figure 2. Plant biomass C (**A**) and plant biomass N (**B**). *Different letters above/below bars* indicate significant differences ($P \leq 0.05$; Tukey's test) in pools at the different times of harvest. Note that the time between each bar on the *X*-axis is 1 month except between November and April (5 months). *Error bars* represent 1 standard error, $n = 12$.

opposite trend (Figure 3C). Organic N forms dominated the total dissolved N pool in the soil, that is, the DON to inorganic N ratio averaged 44 over the study period.

^{15}N Turnover in Soil and Plant Pools

We have no data on natural abundance of amino acids at our site, but the amount of ^{15}N-labelled glycine added corresponded to around 15% of TDN in October. Sorensen and others (2008) found a similar fraction of ^{15}N-labelled glycine to TDN in a comparable ecosystem. The authors reported that their amount of added ^{15}N-labelled glycine (128 mg ^{15}N m^{-2}) immediately after addition made up about 99.8% of the soil glycine-N pool and 90.8% of the total soil amino acid pool.

Over the full study period, the mean total recovery of ^{15}N tracer above- and belowground was 104 ± 6% of the added amount. The ^{15}N tracer recovery across all harvests was comparable in the microbial biomass and the soil immobilized N fractions (~45% each) with much smaller proportions in the roots (~8%) and TDN (~1%), and aboveground biomass (~0.6%) (Figure 4A). However, the ^{15}N tracer concentration (Figure 4B) was 40–60 times higher and more variable in the microbes than in the soil immobilized N fraction. Because the MBN pool did not change significantly over the study (Figure 3A), the pattern of microbial ^{15}N tracer concentrations (Figure 4B) reflects uptake/release of the tracer. Accordingly, soil microbes immobilized 65 ± 7% of the added tracer by the first harvest in October (Figure 4A), but released almost half of that by early April (Figure 4B) before the soil temperatures indicated soil thaw (Figure 1). Tracer recovery in the total dissolved nitrogen pool (TDN) was significantly higher immediately after its addition in October than at the other harvest dates (Figure 4A). Neither the recovery of tracer ^{15}N nor its concentration in the total root biomass changed significantly over the study, but both variables in the total aboveground biomass increased slightly by the final harvest in June (Figure 4A, B).

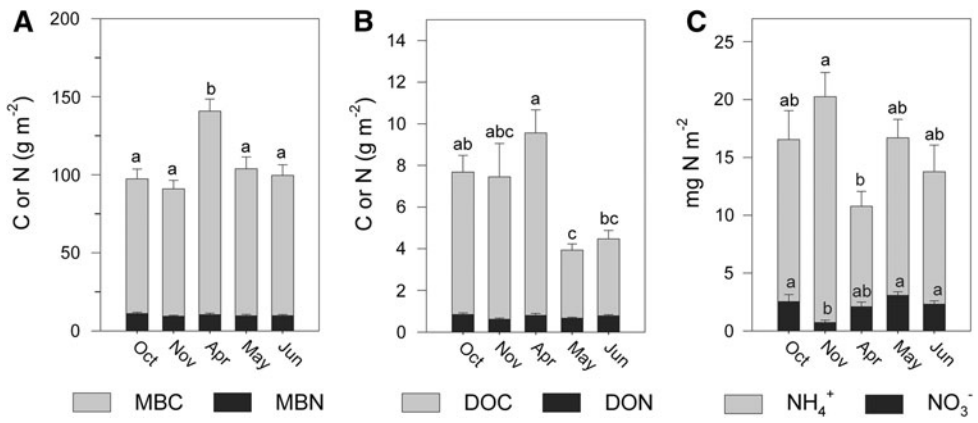

Figure 3. Microbial biomass C (MBC) and microbial biomass N (MBN) (**A**), dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) (**B**), and dissolved inorganic N pools (**C**). *Different letters above bars* indicate significant differences ($P \leq 0.05$; Tukey's test) in individual pools at the different times of harvest. Note that *bars* represent individual pools (non-stacked) and that the time between each bar on the *X*-axis is 1 month except between November and April (5 months). *Error bars* represent 1 standard error, $n = 12$.

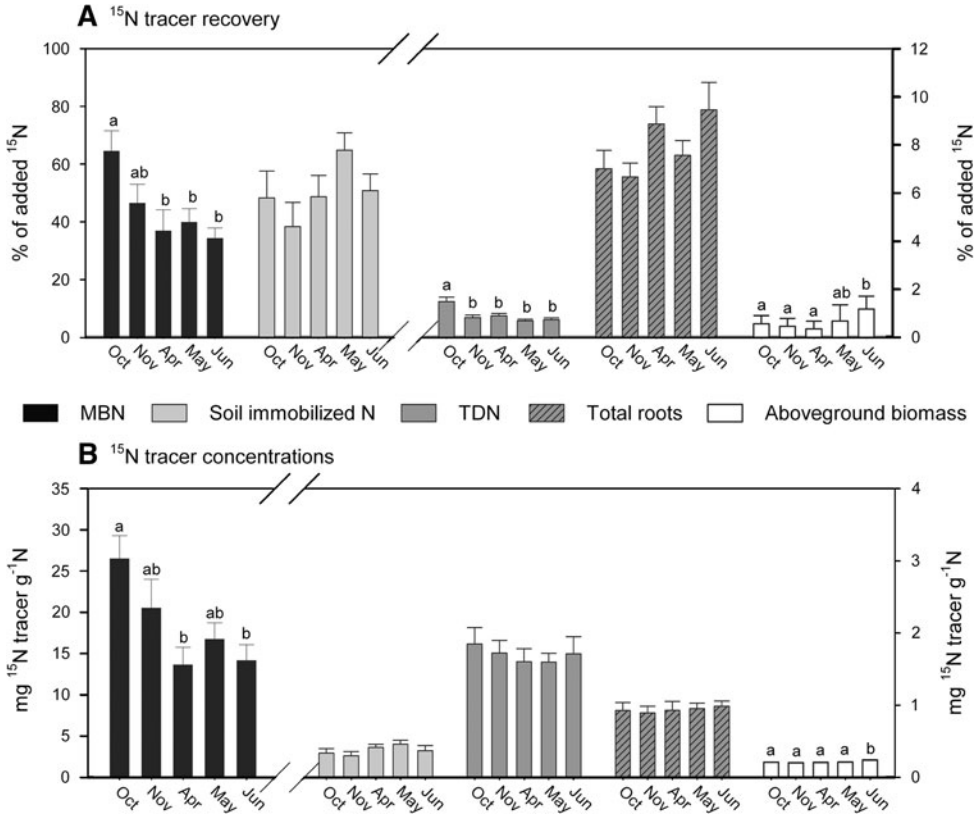

Figure 4. Partitioning of added ^{15}N-label shown as ^{15}N tracer recovery in percent of addition (**A**) and as ^{15}N tracer concentration relative to the total N mass in each pool (**B**). MBN is microbial biomass N and TDN is total dissolved nitrogen. Soil immobilized ^{15}N is the added label recovered in the soil samples minus the ^{15}N tracer content of MBN and TDN. *Different letters above bars* indicate significant differences ($P \leq 0.05$; Tukey's test) in pools or concentrations at the different times of harvest. *X*-axis breaks indicate which *Y*-axis should be read for the different pools. Note that the time between each bar on the *X*-axis is 1 month except between November and April (5 months). *Error bars* represent 1 standard error, $n = 12$.

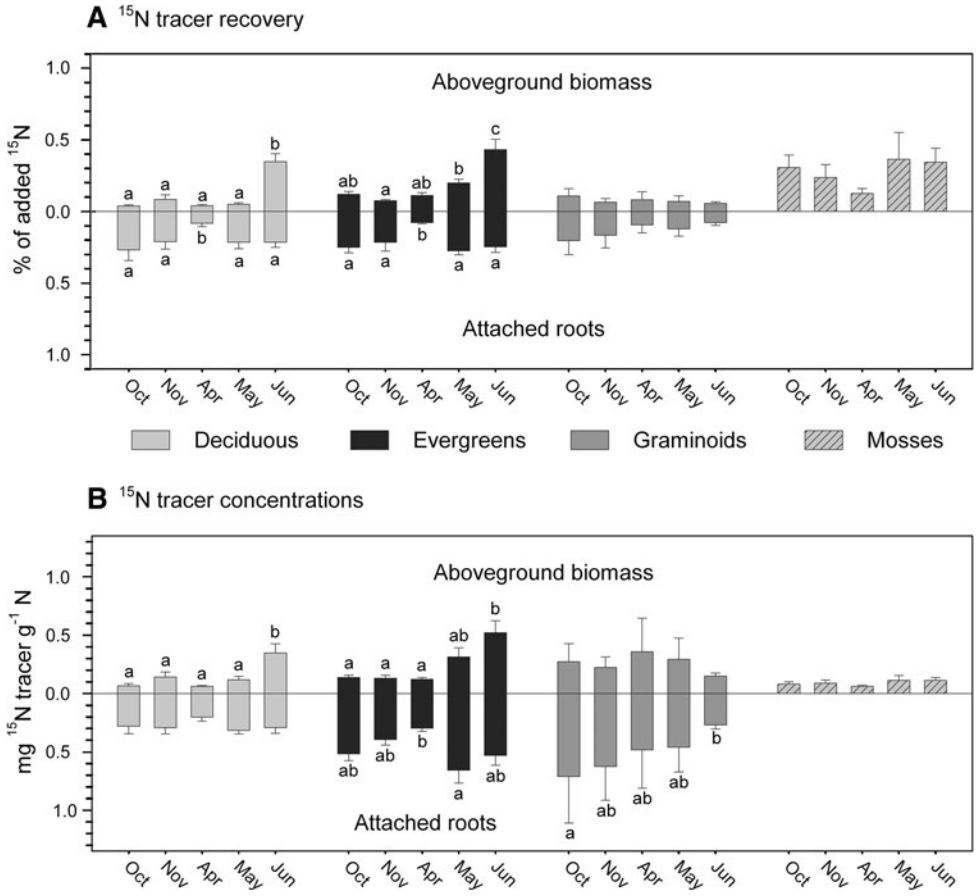

Figure 5. Partitioning of added ^{15}N-label among plant functional groups shown as ^{15}N tracer recovery in percent of addition (**A**) and as ^{15}N tracer concentration relative to the total N mass in each pool (**B**). Root biomasses and fractions here only include roots that were still attached to aboveground biomass after excavation. *Different letters above/below bars* indicate significant differences ($P \leq 0.05$; Tukey's test) between pools or concentrations at the different times of harvest. Note that the time between each bar on the *X*-axis is 1 month except between November and April (5 months). *Error bars* represent 1 standard error, $n = 12$.

The ^{15}N tracer recovery in attached roots of both deciduous and evergreen shrubs was lower in April than in any of the other months (Figure 5A), but as for the biomass C and N of these pools (see above) this was caused by the smaller soil volume excavated at this time due to frozen hard soil. The ^{15}N tracer concentration in the evergreen shrub roots showed a tendency to decrease from October to April but then increased significantly from early April to early May (Figure 5B) indicating active nitrogen uptake during this period. This increased uptake resulted in about 3 times higher ^{15}N tracer concentrations in the evergreen shoot biomass by late June compared to April (Figure 5B). Shoot ^{15}N tracer concentrations in the deciduous shrubs also increased from May to June, but to a lower magnitude (~2 times) and uptake began significantly later (after early May) (Figure 5A). In contrast, the ^{15}N tracer recovery in graminoids tended to decrease slowly and almost linearly with time after the first harvest, particularly in the roots (Figure 5A) and, also contrary to the dwarf shrubs, the ^{15}N tracer concentration was significantly lower by June than in October (Figure 5B). The ^{15}N tracer recovery in mosses was constant over the whole experimental period. For all plant functional group fractions we also calculated ^{15}N tracer concentrations on a dry mass basis and found identical results (data not shown) indicating that changes in plant biomass N concentrations during the study period were minimal and therefore do not affect our interpretation of the results.

Discussion

Using ^{15}N-Labelled Glycine as Tracer

The fertilization effect of the added amount of ^{15}N-labelled glycine in our study (129 mg ^{15}N m^{-2}) was most likely not important for our interpreta-

tion of the results. For example, TDN and microbial biomass N pools were around 6.5 and around 86 times larger in October than the added ^{15}N. However, the glycine addition was most likely a major pulse compared to the total soil amino acid pool assuming a similar fraction of added glycine to total amino acids as reported by Sorensen and others (2008) from a comparable ecosystem (90.8%). Recently, Hobbie and Hobbie (2012) argued that most of the extractable amino acids in soils are unavailable for microbial attack because experiments show that soil microbes take up amino acids within minutes to hours even at concentrations much lower than concentrations usually observed in soil water extracts. The fast soil microbial uptake of available amino acids supports the use of an amino acid as tracer in our study as we aimed to look for increasing tracer recovery and concentrations in plants progressively over time (1–10 months) following a single tracer addition event. This would have been more difficult if a higher proportion of tracer would have entered the investigated plant pools shortly after addition. In support of Hobbie and Hobbie (2012), Sorensen and others (2008) recovered approximately 70% of the added glycine ^{15}N in the microbial biomass 1 day after addition, whereas we found around 65% of added glycine ^{15}N in the microbial biomass 1 month after the addition. In both studies, most of the remaining label was recovered in the soil immobilized N fraction. Following Hobbie and Hobbie (2012), a fraction of the added label was therefore either quickly mineralized by soil microbes (before 24 h) and then released and tied up in soil immobilized N or directly chemically protected in the soil or tied to microaggregates in soil pores inaccessible to microbes.

Hobbie and Hobbie (2012) raised concerns on how to interpret labelled amino acid addition studies, especially with respect to short-term uptake kinetics and the apparent uptake of intact amino acids in plants. Especially with respect to the latter, Warren (2009) showed that substrate concentrations greatly affected plant preferences for different N forms. In the present study, however, we make no conclusions with respect to uptake of intact amino acid in plants and we draw no conclusions with respect to the actual rates of N loss or uptake in the various pools. We base our conclusions primarily on the timing of changes in tracer recovery and concentrations between the first and last harvest. Finally, as our experiment represents a progression over time since tracer addition the temporal pattern of pool dilution could potentially bias the results. Potentially, a large uptake of tracer immediately after addition could cause tissue tracer concentrations (that is, g ^{15}N g^{-1} N) to increase to a level that later can become higher than tracer concentrations in the primary N uptake pool. In such a scenario active N uptake would actually lead to decreasing plant tracer concentrations. In our experiment, however, the tracer concentration in both microbial biomass (the major source of newly plant-available mineral N) and the TDN pool consistently stayed much higher than tracer concentrations in the plants. Active plant N uptake concomitant with decreasing plant tissue tracer concentrations therefore seems unlikely in our study.

Microbial C and N Turnover

The relatively few investigations focusing on unravelling the significance of plant activity outside the growing season in the Arctic contrasts with the vast number of investigations over the last 20 years of soil microbial activity and community dynamics during the cold season. Some of these studies suggest that the structure of the entire soil microbial community during winter may be different from that during summer (for example, Schadt and others 2003; Lipson and Schmidt 2004), but most studies have focused on changes in total microbial biomass between summer and winter. Our microbial biomass C results correspond to the generally observed pattern of peak microbial biomass in late winter followed by a major decline during snowmelt (for example, Brooks and others 1998; Larsen and others 2007a; Jefferies and others 2010; Buckeridge and Grogan 2010). Similar to Brooks and others (1998), we did not find significantly increased inorganic soil N concentrations with the decline in microbial biomass C. However, microbial biomass N and microbial ^{15}N tracer recovery indicated a different pattern than observed for microbial biomass C. The decrease in ^{15}N tracer recovered in the microbial biomass N from October to April indicates a major loss of microbial nitrogen during the winter, whereas there was no further reduction in microbial ^{15}N tracer during the subsequent snowmelt period. Many incubation studies have shown that repeated freezing and thawing of the soil reduces the microbial biomass (for example, Ross 1972; Schimel and Clein 1996; Larsen and others 2002), and observed changes in microbial biomass during snowmelt have often been attributed to freeze–thaw events (Brooks and others 1998; Jaeger and others 1999). It has also been suggested that the changes in carbon availability and temperature, and not freeze–thaw

cycles, may be the main factors controlling the changes in the microbial community and the size of the biomass in the winter–summer transition (Lipson and others 2000). Recently, Jefferies and others (2010) suggested that the change in soil water osmotic potential following a sudden flux of melt water rather than changes in soil temperature and/or freeze–thaw events itself, is the primary cause of late winter/early spring microbial dieback.

Although all these studies have focused on the microbial dieback during spring thaw, our results indicate that the winter period, despite increasing microbial C and constant microbial N pools, may be more important for N turnover than indicated by total C and N pools alone. It is possible that the relatively warm weather front in late February, which most likely led to snowmelt and a flux of melt water down into the soil, followed by a cold period with soil temperatures rapidly cooling down to −12°C (Figure 1) caused a dieback in the winter microbial community. Surviving microbes, possibly dominated by fungi over bacteria as indicated by the increased microbial C to N ratio, then recovered and peaked in April, before crashing again during spring thaw. This could explain both the changes in microbial C and microbial ^{15}N tracer recovery. In any event, although the mechanisms remain unclear, our microbial ^{15}N results indicate substantial microbial N turnover by early April, long before the springtime periods of snow melt, soil thaw and freeze–thaw events.

Plant N Uptake During the Cold Season

Our results support the hypothesis that some arctic plants are capable of nitrogen uptake during late winter/early spring, but not beforehand. Active N uptake by the evergreen dwarf shrubs took place between early April and May, when mean soil temperatures were still below 0°C for most of the time. This conclusion is based on the significant increase in ^{15}N tracer concentration observed in evergreen roots from April, when it was lowest of all times, until May. As mentioned, the April soil excavation was difficult due to frozen hard soil. If roots had lower tensile strength at this time it is possible that we obtained a higher fraction of coarse roots to fine roots during root sorting. Because the highest ^{15}N tracer concentrations are expected to be found in the fine roots this could have biased the results towards a lower total root ^{15}N tracer concentration. We find it more likely, however, that the low ^{15}N tracer concentration in the evergreen roots at the April harvest coincided with the annual minimum of fine root biomass just before the end of winter and that this caused a higher fraction of coarse to fine roots and thus a low total root ^{15}N tracer concentration. Secondly, aboveground evergreen biomass also tended to increase in ^{15}N tracer concentration from April to May thus supporting active uptake by the roots. In comparison to our study, Bilbrough and others (2000) found significant nitrogen uptake by alpine perennial forbs and graminoids during the spring snowmelt, whereas all arctic plant functional species, including evergreen and deciduous shrubs, showed a much lower uptake potential under the snow (<1% of annual nitrogen uptake). They suggested that the low uptake in arctic plants could have been due to much more severe mid-winter soil temperatures (−14.5°C) at that site compared to −8°C at the alpine site. In our study, soil temperatures were around −5°C for most of the winter but did reach −12°C in late February, which is comparable to the arctic site in the study by Bilbrough and others (2000). Nevertheless, the evergreen shrubs at our site had taken up nitrogen only 10 days after snowmelt was completed. It has often been suggested that the post-snowmelt period is of high importance to plant nutrient uptake, but the present study is, to our knowledge, the first in situ experiment that has actually shown this for an arctic heath ecosystem. In agreement with our hypothesis, our results indicate that it is primarily the evergreen dwarf shrubs rather than the other functional plant groups that are capable of N uptake at this time of year.

Considerable plant nitrogen uptake during fall, as well as high microbial release of nitrogen over the following winter–summer transition concurrent with increased plant uptake, has previously been shown in the understory of a subarctic birch forest (Grogan and Jonasson 2003). In their study, no plant nitrogen uptake was observed over the cold season (October to late May), whereas significant ^{15}N uptake by all functional plant groups took place during the growing season. The higher temporal resolution of the late winter measurements in our study shows a more detailed pattern of nitrogen uptake by the plant functional groups during the snowmelt period. The evergreen dwarf shrubs were able to take up nitrogen immediately at the onset of soil thaw in April–May, and they rapidly allocated it to aboveground tissues. In contrast, the uptake and allocation to aboveground tissue by deciduous dwarf shrubs was delayed to the May–June period, whereas the graminoids had not increased their ^{15}N tracer pool by June in comparison to the October pool. The uptake of ^{15}N tracer by the mosses was likely restricted due to the

lack of roots and the fact that the tracer was added primarily to the soil.

Our results indicate a temporal sequence of N uptake and allocation among the functional plant groups with the evergreen shrubs being active as soon as the soil thaws, followed by the onset of N uptake by the deciduous shrubs, and later by the graminoids (which had highest uptake during fall). This coincides with a report of earlier onset of fine root production and earlier re-greening of leaves in Alaskan evergreens shrubs as compared to deciduous dwarf shrubs (Kummerow and others 1983) as well as the reported significant photosynthetic activity during snowmelt in evergreen shrubs found by Starr and Oberbauer (2003). As the summer proceeds, it appears, however, that the faster-growing deciduous shrubs and herbs increase their rate of N uptake and reach the same level of N acquisition as the evergreens, judged from July data by Grogan and Jonasson (2003). This could also explain the lack of differences in the N acquisition pattern in leaves of different longevity in their study. The uptake in early spring may give the evergreen species an advantage that compensates for their generally slower growth rates during summer compared to the deciduous shrubs and graminoid/herbaceous species (Lambers and others 1998). The ability for early nutrient acquisition by the evergreens may also play an important role in maximizing ecosystem primary production through fuelling the substantial carbon uptake observed in the plots during the April–May period, which makes up about 12–14% of annual gross photosynthesis at the site (Larsen and others 2007b).

Conclusions

The microbial biomass C in our study followed a seasonal pattern often observed in arctic ecosystems with peak biomass during late winter prior to spring thaw followed by a significant reduction during spring thaw indicating rapid microbial turnover. In contrast, the microbial biomass lost a significant amount of added ^{15}N tracer during mid-winter indicating that a substantial microbial N turnover is uncoupled from the spring thaw decrease in microbial biomass C. The significant plant uptake of N during spring in this subarctic heath ecosystem coupled with observations of significant cold-season photosynthesis indicate that some arctic plants, especially the evergreens, are active at least during late winter/early spring whereas we found no evidence of N uptake during the earlier months (November–April). Our results suggest a temporal sequence of the resumption of N uptake among the different functional groups with evergreen dwarf shrubs having the highest potential for early N uptake, followed by deciduous dwarf shrubs and graminoids.

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