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CONTROLS ON ANNUAL NITROGEN CYCLING IN THE UNDERSTORY OF A SUBARCTIC BIRCH FOREST

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Abstract. Characterization of the controls on annual nitrogen (N) cycling is critical to understanding the functioning of high-latitude ecosystems and to predicting their responses to perturbations. Here, we describe an experimental evaluation of the effects of season and vegetation on annual N cycling in the understory heath vegetation of a subarctic birch forest. Our approach was to follow the partitioning of an isotopically enriched $^{15}NH_4Cl$ addition between microbes, plants, and the soil solution at intervals through winter, and in the following summer. To investigate the direct influence of vegetation on ecosystem N cycling, the isotope was added to control plots and to plots from which plants had been removed early in the previous growing season.

Our results indicate that the dynamics of both microbial carbon and N were similar in treatment and control plots, suggesting that the presence of intact plants had negligible influence on seasonal patterns of microbial growth or N accumulation in the soils of this ecosystem. Instead, ecosystem N cycling was dominated by a substantial turnover of recently acquired N from microbes during the winter–summer transition that corresponded to a significant increase in understory plant ^{15}N uptake.

Vascular plant ^{15}N uptake and accumulation in belowground tissue was substantial at the onset of winter, but ceased once full winter conditions developed. By contrast, there was a rapid resumption of vascular plant N uptake and strong allocation to aboveground tissue at the onset of summer. Vascular plant types varied strongly in ^{15}N enhancement, depending on physiological differences in N uptake capacity as well as differences in biomass. In particular, *Vaccinium vitis-idaea*, *Vaccinium myrtillus*, and the herbs exhibited strong ^{15}N acquisition capacities, despite their relatively low biomass. We found no consistent evidence that the evergreen vs. deciduous leaf habit contributed to species differences in N uptake capacity. Species capacity for allocation of ^{15}N to new shoot tissue was closely correlated with aboveground production per unit total N and tended to be greatest in herbs and *V. myrtillus*, suggesting that inherent physiological capacities for N uptake and tissue allocation are important determinants of species productivity.

Our results suggest a hierarchy of controls on annual N cycling. The importance of wintertime influences on annual ecosystem N cycling was indicated by ongoing net mineralization by microbes beneath snow cover and a critical N release from microbes during the winter–summer transition. By contrast, plant N uptake was confined to the snow-free season. Thus, seasonal environmental changes caused a rapid microbial turnover of recently acquired N that then became available for plant uptake in the subsequent growing season. Although vascular plant species appeared to compete strongly with each other to acquire this N, neither their presence nor their total N uptake appreciably affected microbial N-cycling activity. Together, these results suggest that season exerts primary control on annual N cycling in this ecosystem, and that its effect on microbial N turnover is a major process supplying N to plants. By comparison, the effects of understory vegetation on N cycling were small within the time frame of this experiment.

Key words: annual N cycling; birch forest; heath; microbial N cycling; ^{15}N isotope; productivity; seasonal effects; species traits; spring thaw; subarctic; understory vegetation; Vaccinium *spp.*

INTRODUCTION

Plant growth in both temperate and high-latitude terrestrial ecosystems is most commonly limited by nitrogen (N) availability (Shaver and Chapin 1980, Schlesinger 1991, Vitousek and Howarth 1991). Thus, characterization of the mechanisms contributing to the maintenance of N limitation is critical to understanding the functioning of many ecosystems (Vitousek et al. 1998). Furthermore, understanding the controls on N availability to plants, on plant N uptake capacity, and on ecosystem N losses is essential to predicting ecosystem responses to perturbations.

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Season exerts strong control over biogeochemical cycling in the Arctic (Dowding et al. 1981, Giblin et al. 1991, Weih 1998, Grogan and Chapin 1999). However, although it has been assumed that negligible biogeochemical cycling occurs in the Arctic during winter, several recent studies indicate considerable biological activity beneath snow cover. Respiratory release of CO_2 occurs throughout the winter at many high-latitude and high-altitude locations (Sommerfeld et al. 1993, 1996, Zimov et al. 1996, Oechel et al. 1997, Winston et al. 1997, Fahnestock et al. 1998, Grogan et al. 2001), albeit at rates 4–10% of those in summer (P. Grogan, *unpublished data*). A recent experimental manipulation indicated that midwinter ecosystem CO_2 production in a heath tundra site was dominated by respiration of plant-associated (i.e., recently photosynthesized) carbon, rather than bulk soil organic matter (Grogan et al. 2001). In addition, active N accumulation in soil microbes has been observed throughout the winter beneath alpine snowpacks (Brooks et al. 1998, Lipson et al. 1999). Tundra litterbag studies indicate that most mass loss and N loss occur within the winter and spring thaw periods (Hobbie and Chapin 1996). Together, these results suggest that active decomposition of fresh plant litter by cold-tolerant soil microbes occurs beneath snow cover in many high-latitude ecosystems.

Annual patterns of N supply in both arctic and alpine soils are characterized by a major increase in soluble N pools at spring thaw (Dowding et al. 1981, Brooks et al. 1996, 1998, Schimel et al. 1996, Lipson et al. 1999). Microbial N pools generally exceed those of total soil solution N in both high-latitude and high-altitude ecosystems (Giblin et al. 1991, Nadelhoffer et al. 1992, Schmidt et al. 1998, Jonasson et al. 1999, Lipson et al. 1999) indicating that even relatively minor fluctuations in the former pool can have major impacts on N availability to plants. The spring thaw N flush in these ecosystems is attributed to springtime wet-up and freeze–thaw events that cause microbial lysis and release of cellular contents into the soil solution (Ivarson and Sowden 1970, Skogland et al. 1988, Schimel and Clein 1996). Nitrogen inputs from melting snowpacks tend to be minor in arctic ecosystems because total accumulation of atmospheric N deposition over winter is low compared to typical N fluxes in the soil during spring thaw (Schimel et al. 1996). In contrast to the winter and spring thaw period, net N mineralization rates in summertime tend to be relatively low, and net microbial N immobilization during the plant growing season is common (Giblin et al. 1991, Nadelhoffer et al. 1992, Schmidt et al. 1998). Together, these studies suggest that wintertime N cycling has the potential to have strong effects on the annual pattern of ecosystem internal N transfers in arctic and alpine ecosystems.

Vegetation exerts significant control on nutrient cycling through the quantity and quality of litter production by plant species, impacts on soil microenvironment, and through the influence of the rhizosphere on soil biogeochemical processes. Plant roots support nutrient-acquiring mycorrhizal associations (Read 1991) and exude low molecular weight carbon compounds that may prime organic matter decomposition (Clarholm 1985, Cheng and Coleman 1990). Finally, ecosystem N cycling can be strongly influenced by plant species differences in physiological traits such as nutrient uptake capacity, degree and type of mycorrhizal association, and leaf tissue chemistry and longevity, e.g., evergreen vs. deciduous leaf habit (Chapin 1980, Hobbie 1992).

In this study, we contrast the relative influences of season and vegetation in controlling annual patterns of nitrogen availability to plants in a subarctic heath ecosystem. In addition, we investigate the importance of wintertime N cycling to annual plant production in high-latitude ecosystems. What is the fate of N accessed by microbes during winter? Can plants directly compete for soil N during winter? If climate changes result in increased soil decomposition activity, would the additional N mobilized by microbes ultimately be acquired by plants or lost from the ecosystem as leachates or gases? To our knowledge, this is the first study to address these questions by following the fate of isotopically enriched ^{15}N label additions in plant, microbial, and soil solution pools at regular intervals through an arctic winter, as well as in the following summer. We tested the following hypotheses: (1) The presence of plants substantially influences the annual pattern of microbial N cycling in a high-latitude ecosystem. (2) Microbial N release during the winter–summer transition is a critical process in supplying N to plants and in determining potential ecosystem losses. (3) The evergreen leaf longevity trait confers additional plant N uptake capacities at the very beginning and end of the growing season at high latitudes.

Materials and Methods

Site description

This study was conducted on a gentle slope within the understory heath vegetation of a birch forest (*Betula pubescens* ssp. *tortuosa* Ledeb.) near Abisko (68°20′42″ N, 18°50′18″ E; ~415 m above sea level) in northern Sweden. The climate is subarctic, with mean summer and winter temperatures of 10°C and −9°C, respectively, precipitation totaling ~300 mm, and a snow-covered winter season usually lasting from early October to late May (Royal Swedish Academy of Sciences, Abisko Scientific research station).[4] The aboveground biomass and annual shoot production of the polycormic (multi-stemmed) birch trees in this region are ~960 g/m^2 and ~140 g·m^{-2}·yr^{-2}, respectively (Bylund and Nordell 2001). At our site, their density was ~8/100 m^2. The understory heath vegetation is dominated by the evergreen *Empetrum hermaphroditum* Hagerup., *Vaccinium vitis-idaea* L., and the de-

[4] URL: ⟨http://www.ans.kiruna.se/ans.htm⟩

ciduous *Vaccinium myrtillus* L. and *Vaccinium uliginosum* L. In addition, considerable moss cover (predominantly *Hylocomium splendens* {Hedw.} Br. Eu.) occurs, along with occasional lichens. The mass of surface litter and standing dead material is ~340 g/m^2 (P. Grogan, *unpublished data*). Soils are highly organic at the surface, commonly overlaying a gleyed podsol above schist rocks. Soil organic layer depth below the transition from the green to brown moss layers varied from 1 to 13 cm.

Experimental design

In June 1999, six areas of similar vegetation (~1.6 × 2.2 m), within 1–2 m from the base of mature polycormic birch trees, were selected across the site. A trench (~15 cm wide) was dug to the underlying bedrock around each area (block) in order to cut all penetrating birch roots, and then was back-filled with the original soil. Two plots (0.9 × 0.9 m) were selected within each block and were randomly assigned as treatment or control. In the treated plots, all aboveground vegetation (including mosses) was gently pulled out by hand, along with as much of the attached belowground stem and root material from the surface soil as possible (without causing excessive disturbance of the soil). Senesced leaf litter that had been trapped within the understory vegetation was separated and replaced on the soil surface of the treatment plots. Afterward, plastic liners (15 cm high) were inserted 5–10 cm into the soil around the edge of each plot to prevent ingrowth of new roots and to prevent soil run-off. Similar liners were inserted into control plots. Occasional regenerating shoots in the treatment plots were removed in October prior to labeling. Shoot regeneration within the treatment plots was extremely rare thereafter. Soil temperature probes were placed at ~3 cm below the green/brown organic matter transition in each plot. Soil temperature in each plot was logged at 2-h intervals and recorded as 12-h means during winter (October to May), and at every 0.5 h and recorded as 4-h means thereafter.

^{15}N isotopic labeling

Over the period 5–8 October 1999, a solution containing >98% enriched $^{15}NH_4Cl$ (Cambridge Isotope Laboratories, Andover, Massachusetts, USA) was injected into the central 0.72 × 0.72 m of each plot at a rate equivalent to 37.3 mg $^{15}N/m^2$. The label was injected evenly in 5-mL volumes onto the soil surface beneath any fresh litter using a grid frame (324 injections per plot). In control plots, the vascular plants and green mosses were gently pushed aside at each injection location so that the label could be delivered directly onto the soil surface. Control and treatment plots within the same block were labeled on the same day.

Sampling protocol

On 12 October 1999, 11 January, 4 March, 15 May, and 14 July 2000, a randomly located intact sample (0.20 × 0.20 m) of vegetation plus the underlying organic soil was collected within each control and treatment plot. Samples were cut out using a handsaw to a depth of 5–10 cm below the green/brown organic matter transition, and all vegetation rooted within the sample was included. Sample depth varied according to the extent of underlying compacted frozen soil, as well as local variations in the proximity of bedrock or the mineral gleyed soil layer. We recorded the organic layer depth of each sample in order to account for this variation when calculating the bulk densities of soil organic matter in each plot. When snow was present, it was gently brushed aside from the sampling area and was replaced once the sample had been taken. Snow depths were measured at four points per block. The winter samples were sealed in plastic bags and immediately transported to the lab and stored at 2°C, unless the soils were frozen hard, in which case they were stored at 5°C. Sample sorting and processing were completed within 2–4 d. The summer (July) samples were stored at 5°C prior to sorting and processing.

Sample processing

The aboveground plant biomass of each control sample was sorted into *E. hermaphroditum*, *V. vitis-idaea*, *V. myrtillus*, *V. uliginosum*, herbs (mostly grasses, some *Linnea borealis*), mosses, and lichens. Shoots of the vascular plants were separated into ''new'' (i.e., produced in the most recent growing season) and ''old'' categories on the basis of most recent bud scars, transitions in shoot color, and shoot winter hardening, and the size distributions of leaves within each annual cohort in the case of the evergreens *E. hermaphroditum* and *V. vitis-idaea* (earliest and latest leaves being smaller than those initiated in midseason). The final harvest (July) distinguishes production that occurred within the 2000 growing season, whereas all previous harvests separate production that occurred predominantly in the 1999 growing season. In some cases, it appeared as if shoot meristem activity may have been initiated more than once in a growing season. By contrast, shoots that did not appear to contain active growing tips for the current year were observed occasionally (and were confined to the ''old'' category). Flowers and fruits in the July harvest were included in the ''new'' category. Sorting of wintertime samples of *V. uliginosum* presented particular difficulties, and was based predominantly on color and tissue softness. In cases of doubt, shoots and their tips were consigned to the ''old'' category. Living shoots of *E. hermaphroditum* often contained some attached dead branches that were removed and placed with the bulk soil.

To maximize sorting to the species level, each soil sample was gently teased apart during shoot sorting in order to collect as much of the attached roots and belowground stems as possible. For each species and growth form, roots <1 mm in diameter were classified as ''fine roots;'' larger roots and belowground stems

were grouped as ''coarse roots.'' Living tissues were distinguished on the basis of their structural vigor and color beneath the bark and within the heartwood. In cases of doubt, suspect tissues and all obviously dead material were consigned to the bulk soil. In the midwinter harvests (January–May), collection of fine roots associated with each individual species was abandoned because of the negligible quantities present (except for *E. hermaphroditum* in January).

Having removed all of the biomass identifiable to species, we separated the remaining belowground stems and coarse roots into an ''unidentified coarse root'' category, and any attached fine roots were allocated to an ''unidentified fine root'' category. Samples from the treatment (i.e., plant-removed) plots were processed in the same way. Very occasional shoots emerging in these plots were included in the ''unidentified coarse root'' category. Afterward, the belowground remainder was cut up into chunks ~3 cm in diameter and mixed thoroughly. Subsamples were picked randomly and sorted into ''unidentified fine roots (subsample),'' ''soil organic matter'' (22–30 g fresh mass), and, where necessary, a ''soil bulk remainder'' fraction consisting of occasional remnant fragments of fresh leaf litter and living coarse roots. In order to be consistent, we sorted as much fine root biomass from the ''soil organic matter'' fraction as possible within a 15-min period. Subsamples generally constituted 3–7% of the total belowground bulk sample. Each coarse and fine root sample category was washed twice in water and then immersed in 0.005 mol/L K_2SO_4 for >2 min to remove any adhering ^{15}N label from exterior surfaces (Kielland 1997). The fresh mass values of the soil bulk remainder and of a subsample of the ''soil organic matter'' for each sample were recorded to determine their gravimetric moisture contents. Afterward, these samples were dried along with all other categories at 70°C for 2–5 d. Our results indicate that this sample-processing protocol was effective in sorting ~60% of the total plant biomass to the level of individual plant species.

Because the organic layer depth of the samples varied among plots and among sampling months, all soils and soil solution C and N data and belowground biomass data have been recalculated (on a proportional depth basis) to a standard of 5 cm below the green/brown transition in the moss layer.

Biological and chemical analyses

Soil microbial biomass C and N contents were determined by the chloroform-fumigation direct-extraction technique (Brookes et al. 1985) using 10 g fresh mass of soil (but 4 g in July) and 50 mL 0.5 mol/L K_2SO_4 for each extraction. Fumigation was done with 25 mL ethanol-free chloroform for 24 h at ~21°C in a darkened desiccation jar.

Extractions of the non-fumigated samples were conducted immediately after sorting was completed. All samples were shaken with extractants for 1 h and then were let stand for a further 1 h before filtering (in Whatman D paper, Whatman, Maidstone, Kent, UK). Blanks without sample were included to detect contamination during extraction and filtration. All extracts were transported to Copenhagen and stored in freezers until analyses. NH_4-N and NO_3-N contents in the non-fumigated extracts were determined colorimetrically by the indophenol and sulphanilamide methods, respectively (Allen 1989). C contents in the fumigated and non-fumigated extracts were determined on a TOC analyzer (Shimadzu, Kyoto, Japan). Total N within the extracts was determined by Kjeldahl digestion (20 mL) and colorimetric analysis (Kedrowski 1983). Dissolved total N (DTN) is reported as the total N content of the digested nonfumigated extracts (i.e., soluble organic N plus inorganic N). Microbial biomass C and N values were calculated as the differences between fumigated and nonfumigated extracts (Brookes et al. 1985, Vance et al. 1987), using correction factors of 0.35 (k_C) and 0.40 (k_N), respectively (Jonasson et al. 1996) to account for microbial tissue C and N that is not released by exposure to chloroform. Thus, the same fumigation periods and correction factors have been applied to all microbial data on the assumption that the efficiency of fumigation-extraction remains constant throughout the year. Prior to calculation per gram of soil and per square meter, all C and N concentrations in the extracts were corrected for dilution associated with the moisture content of each soil sample.

^{15}N isotopic analyses

^{15}N label enhancement of the microbial biomass N pool was determined using the acid-trap diffusion technique (Stark and Hart 1996) on the digested extracts. Teflon traps containing two washed and acidified filter paper discs were placed on the surface of the digest solution (80 mL) within specimen cups (185 mL). All non-fumigated samples were spiked with 40 μg N (of natural abundance ^{15}N) to raise the total N contents to the optimum required for isotope-ratio mass spectrometry (IR-MS) analyses. Afterward, 12 mol/L NaOH (18 mL) was added to each cup, before quickly sealing and inverting it. The pH of a random selection of cups was tested to ensure that sufficient alkali had been added to raise the pH >13. The samples were diffused for 8 d, during which time each cup was gently agitated 4–5 times at regular intervals. Afterward, the filter papers were removed and dried in a desiccator jar. Diffused and pipetted standards (1.6297 atom % ^{15}N of total N) were included in each sample batch (Stark and Hart 1996).

^{15}N contents of the filter paper traps, as well as ^{15}N and total N contents of the plant and soil samples were determined by IR-MS at The National Research Institute, Risoe, Denmark. In addition, ^{15}N natural abundances were determined on samples of soils and complete shoots of each species or category that were col-

lected at the time of the July harvest from random locations >4 m from the plots. Mean plant natural abundance ^{15}N isotope atom percentages ranged from 0.3636% (*V. vitis-idaea*) to 0.3654% (herbs); the mean soil value was 0.3660%; the mean soil microbial value was 0.3699%. Isotopic enhancement of the ^{15}N pools arising from the injected ^{15}N label was calculated for each component as

$$\{\text{atom\% } {}^{15}N_{\text{measured}} - \text{atom\% } {}^{15}N_{\text{natural abundance}}\} \times \text{total N pool size.}$$

^{15}N diffusion blank correction was based on isotope dilution (Stark and Hart 1996), after correcting for the isotopic pool dilution associated with the N spike addition to non-fumigated samples. ^{15}N recovery of the pipetted and diffused standards (50–350 μg N) was: $^{15}N_{\text{measured}} = 0.94 \times (^{15}N_{\text{added}}) + 18.74$, $r^2 = 0.99$; and $^{15}N_{\text{measured}} = 0.76 \times (^{15}N_{\text{added}}) + 12.34$, $r^2 = 0.92$, respectively. Total N recovery in ~40% of samples was incomplete because the digestion volume used contained more N than the N uptake capacity of the acid traps (estimated at 350 μg N; Stark and Hart 1996). Accordingly, we used the colorimetrically determined digest N concentrations and the diffusion-determined ^{15}N atom % data to calculate ^{15}N label enhancement in all digested extracts. Stark and Hart (1996) demonstrated that incomplete total N recovery during diffusion does not affect the accuracy of ^{15}N label enhancement determinations, provided that the isotope dilution approach to blank correction is used. ^{15}N label enhancement of the microbial isotopic N pool was calculated using the same k_N factor as for total microbial biomass N. This approach assumes that the incorporated ^{15}N label pool within microbes was in a form that was equally resistant to chloroform fumigation as the total microbial N pool. If the incorporated label were less resistant, then our results would probably overestimate label enhancement of the microbial biomass. Nevertheless, because the same k_N factor was applied to all MBN determinations, the *proportion* of label initially taken up that was subsequently released was unaffected by k_N.

We calculated non-extractable, non-chloroform labile ''soil ^{15}N remainder fractions'' for each sampling month (i.e., abiotic fixation of the label that was not removable by 0.5 mol/L K_2SO_4) by subtracting the sum of microbial and soil solution ^{15}N label enhancement pools from the total soil ^{15}N label enhancement pool (determined by direct isotopic measurement of a dried subsample of the sorted soil used in the extractions). Although this approach yielded sensible soil ^{15}N remainder fractions for May and July (2.7 and 3.4 mg $^{15}N/m^2$, respectively, in control plots), the sum of microbial and soil solution ^{15}N label enhancement pools exceeded that of the ''total soil ^{15}N values,'' indicating apparently negative values (of a similar magnitude to the May and July data) for earlier months. There was no treatment effect. This pattern of variation in estimated abiotic fixation may have arisen because the k_N factor was underestimated in the early winter months, resulting in unrealistically high microbial ^{15}N label enhancement values during that period. In any event, this analysis suggests that the non-extractable, abiotically fixed ^{15}N fraction in our highly organic soils was a relatively small component of total ecosystem ^{15}N; abiotic fixation in clay–mineral dominated soils is much more significant (Hart et al. 1993). In order to be consistent in our calculation of total ecosystem ^{15}N recoveries across sampling months, we have assumed that abiotic fixation was negligible and have calculated recovery as the proportion of total added label that was measured in the sum of plant (all species and types), microbial and soil solution (i.e., DTN) enhancement pools. Our study's major conclusions concerning the importance of the winter–summer transition in mobilizing N are unaffected by this assumption because the change in soil ^{15}N remainder fractions between May and July was minor compared to the corresponding change in microbial biomass ^{15}N.

Statistical analyses

We tested for significant effects of treatment, sampling month, and species using nested ANOVAs. Where treatment effects were analyzed, treatment was nested within block (total stratum df = 11); sampling month and its interaction with treatment were nested within plot, as a substratum within block (total substratum df = 48). Where plant parameters were analyzed within the control plots alone, sampling month was nested within block (total stratum df = 29); species and its interaction with sampling month were nested within plot (total substratum df = 150). Where plant parameters within the control plots were analyzed in July alone, species was nested within block (total df = 29). Significant differences between means were identified using Fisher's Least Significant Difference (LSD) test. Sampling month was treated as a categorical variable. In all data analyses, every statistically significant effect and interaction ($P < 0.05$) is reported in the text. Percentage data (i.e., ^{15}N allocation of new shoots, and total ecosystem ^{15}N recovery) were arcsine square-root transformed prior to analyses (Zar 1996). Lichens were not included in any of the statistical analyses because they formed a very minor component of ecosystem biomass and N pools.

RESULTS

Seasonal and plant effects on environmental conditions

The annual pattern of mean diel soil and air temperatures indicated strong thermal insulating effects of wintertime snow cover (Fig. 1). Soil temperatures were close to 0°C for most of the winter (Oct.–May), except for a short period at ~−5°C in mid-December. Once snow accumulation exceeded ~30 cm (mid-January to

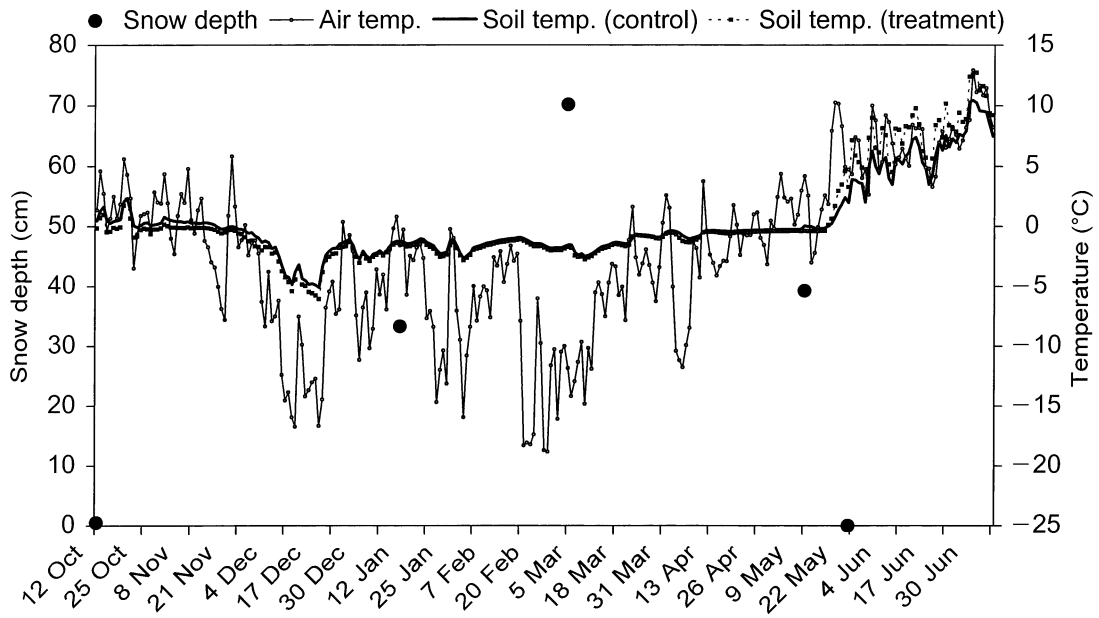

FIG. 1. Snow depths and mean diel soil temperatures in the experimental plots from winter 1999 to summer 2000. Mean diel air temperatures from a nearby weather station are also indicated.

mid-May), soil temperatures remained relatively constant, despite strong fluctuations in air temperature (Fig. 1). The plant-removal treatment had no effect on soil temperature during winter but did result in marginally warmer soils during summer (Fig. 1). Soil moisture in the uppermost 5 cm was significantly affected by sampling month, treatment, and their interaction (Table 1; Month, $F_{4,40} = 103.2$, $P < 0.001$; Treatment, $F_{1,5} = 5.86$, $P = 0.06$; Interaction, $F_{4,40} = 3.04$, $P = 0.028$; $LSD_{\text{within treatments}} = 71$). Soils became increasingly wet through the winter, culminating in saturating conditions beneath the melting snowpack in May (Table 1). Rapid drying after the spring thaw resulted in minimum levels of soil moisture by mid-July (Table 1). The higher soil moisture in control plots during midwinter is most likely a post-harvest artifact due to the melting of overlying snow trapped within the sample vegetation. The soil bulk density was slightly higher in late winter/summer as compared to early winter (Table 1; $F_{4,40} = 5.55$, $P = 0.001$; $LSD_{\text{within treatments}} = 0.016$), possibly due to a compaction effect of the accumulating snow, but was not significantly affected by treatment.

Seasonal and plant effects on annual carbon and N cycling

Biomass components.—The plant-removal treatment in the previous summer strongly reduced coarse root biomass (Fig. 2; $F_{1,5} = 30.25$, $P = 0.003$) and led to a moderate reduction in fine root biomass ($F_{1,5} = 7.21$, $P = 0.044$). In addition, fine root biomass in both treatment and control plots was significantly influenced by sampling month ($F_{4,40} = 19.04$, $P < 0.001$), largely due

TABLE 1. Soil moisture, bulk density, and depth of organic soil sample in control and treatment plots through the experiment.

Sampling month	Soil moisture (% dry mass)		Bulk density (g/cm^3) in the top 5 cm		Organic soil depth sampled (cm)	
	Control	Treatment	Control	Treatment	Control	Treatment
October	268^{a} (22)	290^{a} (16)	0.042^{a} (0.007)	0.038^{a} (0.002)	6.8 (0.7)	6.4 (0.5)
January	526^{b} (39)	441^{b} (33)	0.041^{a} (0.008)	0.058^{b} (0.009)	6.4 (0.5)	5.8 (0.5)
March	523^{b} (17)	478^{b} (32)	0.047^{ab} (0.007)	0.057^{b} (0.004)	4.7 (0.7)	5.7 (0.7)
May	626^{c} (29)	554^{c} (30)	0.058^{b} (0.009)	0.064^{b} (0.007)	5.8 (0.6)	5.6 (0.8)
July	191^{d} (13)	192^{d} (11)	0.061^{b} (0.010)	0.065^{b} (0.004)	8.2 (1.0)	7.0 (0.8)

Notes: Parentheses indicate standard errors ($n = 6$). Means within a column that are followed by a different superscript letter are significantly different ($P < 0.05$).

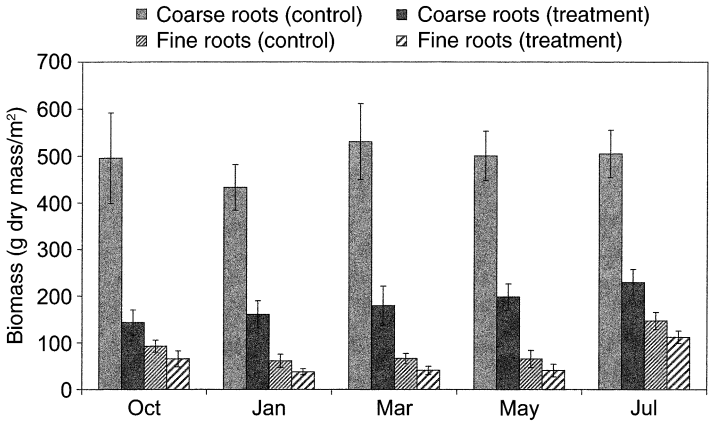

FIG. 2. Seasonal and plant-removal treatment effects on belowground root biomass (mean ± 1 SE) to 5 cm depth.

to enhanced growth after May (Fig. 2; $LSD_{\text{within treatments}} = 31.3$).

Microbial biomass carbon (MBC) was not significantly affected by the plant-removal treatment (Fig. 3), but differed significantly between sampling months (Fig. 3; Month, $F_{4,38} = 8.33$, $P < 0.001$). MBC gradually increased from October to May, and then significantly decreased between May and July (Fig. 3; $LSD_{\text{within treatments}} = 14.0$). By contrast, we observed no significant effects of treatment or sampling time on the microbial biomass N content, MBN (Fig. 3). Thus, it appears that microbial growth continued throughout the winter without N accumulation, resulting in a gradual increase in microbial C:N until May (~10:1), followed by a decline in July (~7:1) (Month, $F_{4,38} = 2.95$, $P = 0.032$; Treatment, $F_{1,5} = 4.19$, $P = 0.096$; data not shown). Together, these results demonstrate that the presence of intact vegetation had negligible impact on soil microbial growth or N accumulation at the 6–12 wk intervals of our successive samplings. By contrast, season had a strong effect in reducing MBC over the winter–summer transition, but had no effect on MBN (suggesting a switch from a fungal-dominated soil microbial community in winter to a more bacterially dominated community in summer).

Soil solution.—The dissolved total nitrogen (DTN) content of the soil solution was significantly influenced by sampling month, treatment, and their interaction (Fig. 4; Month, $F_{4,40} = 5.18$, $P = 0.002$; Treatment, $F_{1,5} = 8.26$, $P = 0.035$; Interaction, $F_{4,40} = 2.94$, $P = 0.030$). DTN increased in the control plots through the winter to reach a peak in May, and then declined strong-

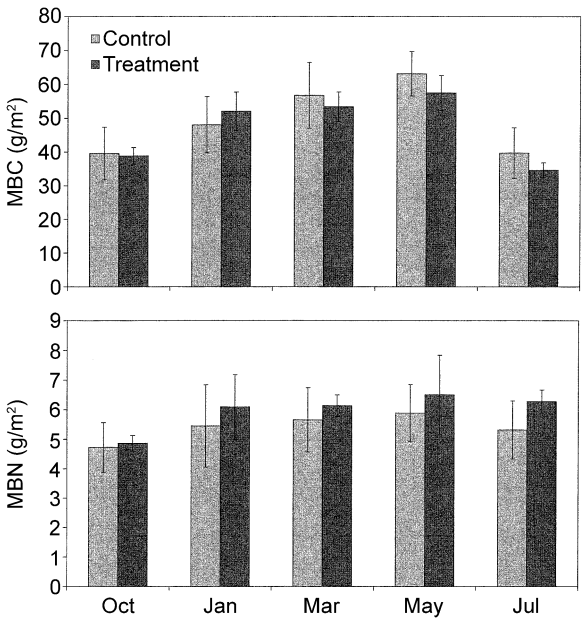

FIG. 3. Seasonal and plant-removal treatment effects on microbial biomass carbon and nitrogen contents (mean ± 1 SE).

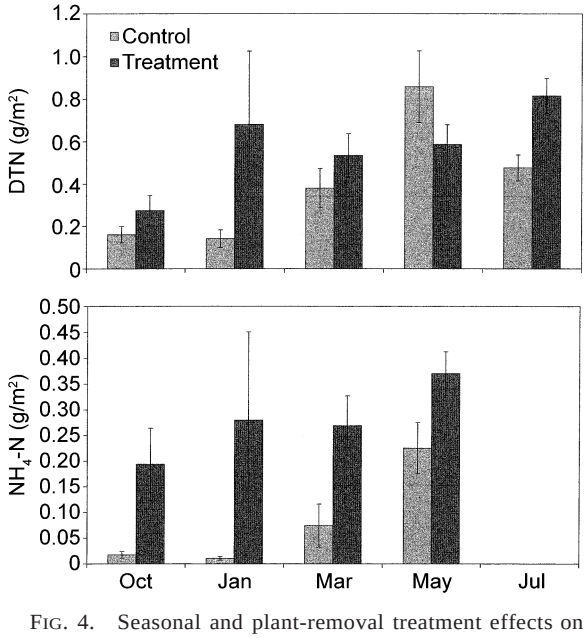

FIG. 4. Seasonal and plant-removal treatment effects on dissolved total N (DTN) and ammonium N (NH_4-N) in the soil solution (mean ± 1 SE). Ammonium data for July are absent because the samples were lost during processing.

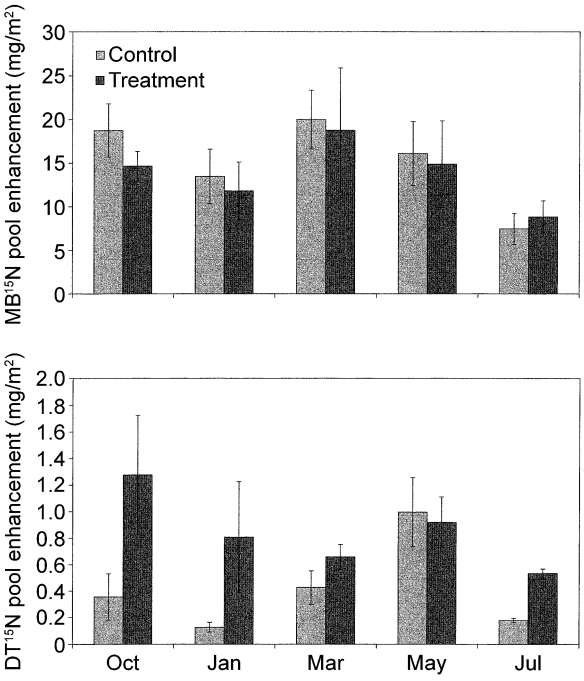

FIG. 5. Seasonal and plant-removal treatment effects on ^{15}N label in the microbial biomass ($MB^{15}N$) and on dissolved total N ($DT^{15}N$) within the soil solution (mean ± 1 SE).

ly by July (Fig. 4; $LSD_{\text{within treatments}} = 0.36$). Plant removal increased DTN for all months except May (Fig. 4). This effect was partially due to corresponding changes in the ammonium component of DTN (constituting 10–25% of DTN in control plots and 30–60% in treatment plots), which was strongly enhanced by the removal of plants (Fig. 4; $F_{1,5} = 19.59$, $P = 0.007$). In addition, there was an overall significant increase in NH_4^+ through the winter months ($F_{3,27} = 3.46$, $P = 0.030$). NH_4^+-N strongly dominated the inorganic N pool: nitrate-N contents were below the detection limit (i.e., <0.01 g N/m^2) throughout the study. In contrast to NH_4^+-N, the dissolved organic N component of DTN tended to increase in control plots through winter, but was generally not affected by treatment (data are not shown). Together, these results indicate a clear pattern of increasing N contents in the soil solution of control plots through the winter up to a peak in May, followed by a sharp reduction over the summer. The plant-removal treatment resulted in strong increases in NH_4^+ within the soil solution at all sampling times. Thus, although understory plants had little effect on overall microbial biomass or microbial N retention (Fig. 3), their removal led to elevated inorganic N in the soil solution during winter (Fig. 4).

^{15}N label partitioning between ecosystem components.—As with total N in the microbial biomass, the plant-removal treatment did not significantly affect ^{15}N label enhancement of the microbial biomass ($MB^{15}N$; Fig. 5). However, in this case, sampling month significantly affected microbial accumulation of the ^{15}N label (Fig. 5; $F_{4,34} = 3.31$, $P = 0.021$). In particular, there was a sharp reduction in $MB^{15}N$ enhancement between May and July for both treatment and control plots (Fig. 5; $LSD_{\text{within treatments}} = 9.1$), indicating a release of ^{15}N from the microbial pool during the winter–summer transition of just over half of the microbial ^{15}N pool. Because the overall N pool within microbes was not significantly affected over the winter–summer transition (Fig. 3), our data indicate that substantial microbial immobilization of unenriched N must have occurred over the same period to counterbalance the MBN loss implied by this ^{15}N release. Furthermore, our study demonstrates that the presence of vegetation had no effect on microbial N turnover during this period, suggesting that it was caused directly by seasonal environmental changes alone.

Consistent with the strong effects of plants on dissolved total N (DTN) and NH_4^+, the ^{15}N enhancement of DTN was significantly higher in treatment than in control plots (Fig. 5; $F_{1,5} = 19.98$, $P = 0.007$). In addition, sampling month had a strong influence on $DT^{15}N$ content ($F_{4,36} = 2.42$, $P = 0.067$). $DT^{15}N$ content increased through the winter in plots where plants were present (Fig. 5), and then sharply declined between May and July ($LSD_{\text{within treatments}} = 0.65$). The seasonal patterns indicate that the presence of plants strongly reduced soil solution $DT^{15}N$ (Fig. 5), and NH_4-N (Fig. 4) in early winter (October–January), but had relatively little effect through the remainder of the winter. Furthermore, the presence of plants led to a sharp reduction in $DT^{15}N$ during the transition to summer. Together, these results strongly suggest that active plant N uptake occurred at the onset of winter as well as early in the following summer.

The complete distribution of added ^{15}N label within the ecosystem (Fig. 6) indicates that soil microbes dominated initial ^{15}N uptake and exerted strong control over its subsequent fate throughout the winter and following summer. Vascular plant ^{15}N uptake during the few days between labeling and harvest in October was substantial, representing ~40% of the total vascular plant uptake by the following July, but was small relative to microbial uptake (Fig. 6). Neither plant nor microbial ^{15}N enhancements changed significantly through the winter. However, there was a sharp, significant increase in vascular plant ^{15}N uptake between May and July (Month, $F_{4,20} = 18.93$, $P < 0.001$; LSD = 0.52) that corresponded with a strong release from the microbial pool (Fig. 6). Together, these results indicate marked seasonal effects on microbial accumulation and subsequent release of recently acquired N, and strongly suggest that plants rapidly accessed some of the released N.

Vascular plant accumulation and translocation of the ^{15}N label depended strongly on sampling month (Fig. 7). Coarse and fine roots accumulated substantial amounts of label during the initial 4–7 d between la-

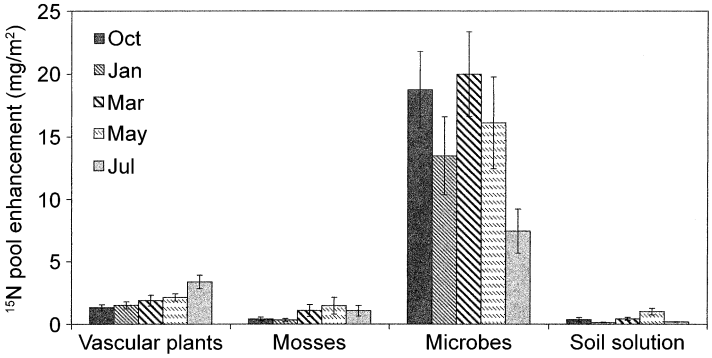

FIG. 6. Distribution of ^{15}N label among ecosystem components within control plots in winter and summer (mean ± 1 SE).

beling and the October harvest (Fig. 7). However, neither total vascular plant nor root ^{15}N contents changed significantly through the rest of the winter (Figs. 6 and 7). Afterward, there were marked increases in ^{15}N in the new and old shoots (Month, $F_{4,20} = 11.80$, $P < 0.001$; LSD = 0.44, Month, $F_{4,20} = 9.33$, $P < 0.001$; LSD = 0.20, respectively) during the transition from May to July (Fig. 7). The absence of a corresponding drop in ^{15}N enhancement in coarse roots over this period (Fig. 7), the drop in ^{15}N enhancement of fine roots (associated with an increase in fine root biomass—Fig. 7), and the strong decline in DT^{15}N between May and July (Fig. 5) together indicate that plant uptake of ^{15}N resumed after May. Vascular coarse roots and old shoots dominated the understory biomass (Fig. 7). This biomass distribution pattern and the absence of seasonal changes in biomass in most of these components (Fig. 7) indicate that plant allocation of the ^{15}N label taken up during summer was specifically directed toward the current year's growing shoot tips. By contrast, the pattern of ^{15}N accumulation by mosses was directly related to the total moss biomass at each sampling

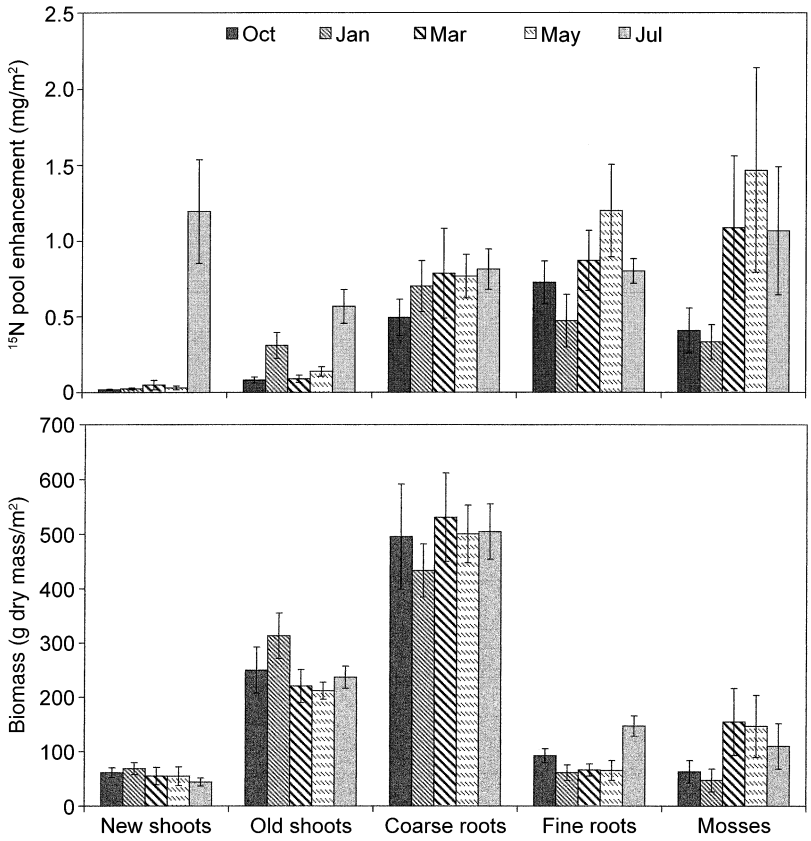

FIG. 7. Distribution of ^{15}N label in plant components within the understory community in winter and summer, and the corresponding seasonal patterns of biomass (mean ± 1 SE). Lichen biomass was minimal (Table 2) and has not been included.

TABLE 2. Biomass, N pool, and production estimates for the understory heath-type vegetation within a subarctic birch forest ecosystem. Parentheses indicate standard errors ($n = 5$; except for production data, with $n = 6$).

Species or plant type	Biomass† (g dry mass/m^2)	N pool† (g N/m^2)	Production‡§ (g dry mass·m^{-2}·yr^{-1})	Production: biomass (yr^{-1})	Aboveground production: total plant N‖ (yr^{-1})
Aboveground biomass					
Vascular shoots					
Empetrum hermaphroditum	138.8 (25.1)	1.08 (0.16)	7.9 (2.6)	0.056	6.0^{a} (1.4)
Vaccinium vitis-idaea	49.8 (7.5)	0.41 (0.05)	4.0 (1.6)	0.080	2.4^{a} (0.8)
Vaccinium myrtillus	46.7 (4.3)	0.49 (0.06)	15.9 (4.0)	0.340	18.4^{b} (3.3)
Vaccinium uliginosum	65.0 (8.1)	0.52 (0.08)	12.7 (5.5)	0.195	10.4^{ab} (1.7)
Herbs	2.7 (0.3)	0.04 (0.01)	3.3 (1.7)	1.222	37.6^{c} (7.3)
Total vascular	303.0 (21.0)	2.54 (0.12)	43.8 (7.4)	0.144	
Nonvascular shoots					
Mosses	104.1 (21.6)	1.22 (0.21)			
Lichens	4.4 (1.8)	0.06 (0.03)			
Total vascular plus nonvascular shoots	411.5 (9.7)	3.82 (0.17)			
Belowground biomass					
Coarse roots					
Empetrum hermaphroditum	65.2 (9.2)	0.31 (0.03)			
Vaccinium vitis-idaea	15.5 (3.4)	0.09 (0.02)			
Vaccinium myrtillus	33.9 (3.8)	0.19 (0.03)			
Vaccinium uliginosum	49.7 (10.4)	0.28 (0.05)			
Herbs	0.5 (0.5)	0.01 (0.01)			
Unidentified	328.3 (18.2)	1.51 (0.10)			
Total coarse roots	493.0 (16.1)	2.39 (0.08)			
Total fine roots	86.3 (16.1)	0.83 (0.11)	85.9		
Total roots	579.3 (25.6)	3.22 (0.14)			
Total vascular plants	882.3 (16.5)	5.76 (0.14)			
Total plants	990.8 (22.7)	7.04 (0.27)			

† Biomass and N pool data for all categories have been computed as mean values over the five sampling times. Aboveground values have been calculated as the sum of ''new'' and ''old'' tissues. Fine root biomass at the species level has not been included because data were available for the October and July harvests only.

‡ Aboveground production values are reported as the means of new shoot production in the period up to the harvest in mid-July and thus are likely to underestimate total annual production. In addition, they do not include stem secondary growth.

§ Total fine root production was estimated by subtracting minimum from maximum biomass values. Belowground production data for individual species are not included because substantial proportions of the stem/coarse root and fine root biomass categories were not identified to species (~67% and ~95%, respectively).

‖ Aboveground production as a percentage of total plant N (i.e., new shoot biomass divided by the total N pool in all shoot tissue and whatever coarse and fine root biomass that was identified to species level). Means followed by a different superscript letter are significantly different ($P < 0.05$).

month (Fig. 7), indicating no seasonal control over N uptake.

Total ecosystem recovery of the added ^{15}N label in plant, microbial, and soil DTN components in control and treatment plots at the first harvest in October was 56% and 44%, respectively (data are not shown), suggesting that there may have been substantial ^{15}N movement down the soil profile during the initial labeling phase. Rain and light snow that fell immediately following labeling may have promoted downward transfer. The plant-removal treatment had no significant effect on ecosystem ^{15}N recovery (data are not shown). However, as expected from the substantial microbial ^{15}N release during the winter–summer transition and the relatively small increase in plant ^{15}N uptake over that period, total ecosystem ^{15}N recovery declined significantly between May and July in both control and treatment plots (Month, $F_{4,33} = 3.37$, $P = 0.020$), to mean values of 32% and 27%, respectively.

Species biomass, N pools, and productivity

The understory heath vegetation was dominated above and belowground by *Empetrum hermaphroditum* (evergreen, woody), followed by mosses, *Vaccinium uliginosum* (deciduous, woody), *V. vitis-idaea* (evergreen, relatively nonwoody), and *V. myrtillus* (deciduous, relatively nonwoody) (Table 2). However, aboveground production up to midsummer, and aboveground production per gram of total plant N (an index of total plant N use efficiency) were considerably higher in the deciduous and herb species than in the evergreens (Table 2). Species variation in N pools broadly correlated with biomass components (Table 2), indicating little variation in tissue N concentrations among species for each component. However, tissue N concentrations were markedly lower in the coarse roots and old shoots (~0.6% and ~0.8%, respectively) than in the fine roots and new shoots (~1.0% and 1.4%, respectively), in-

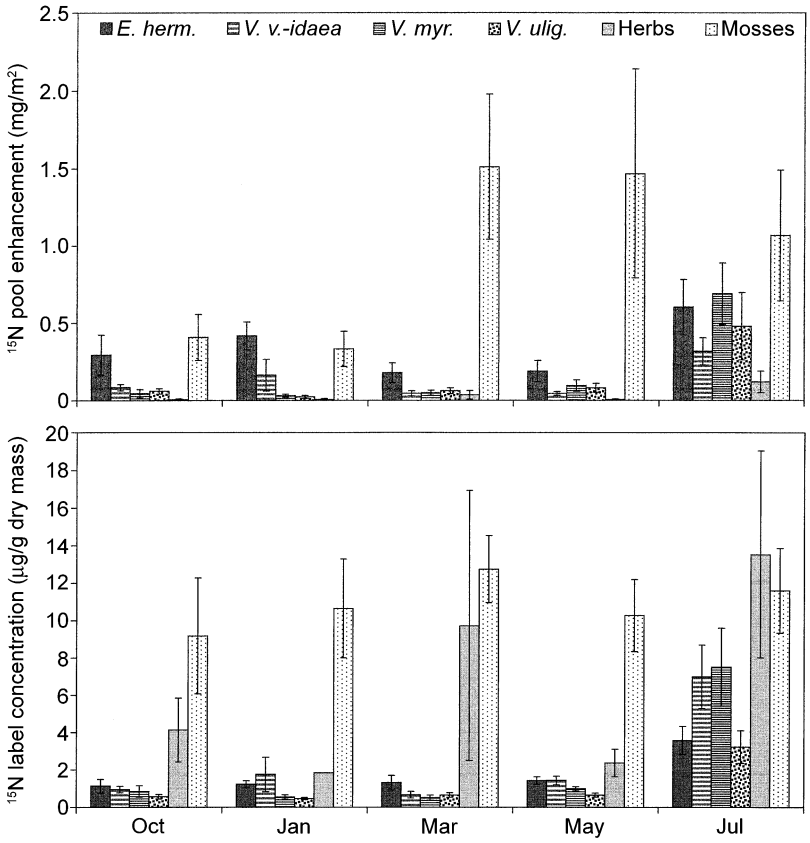

FIG. 8. Total plant ^{15}N label enhancement pool and concentration for each species in winter and summer (mean ± 1 SE).

dicating preferential N allocation to the actively growing meristems. The total understory plant N pool (Table 2) was almost equivalent to the soil microbial N pool (Fig. 3), each representing about one-sixth of the ~30 g N/m^2 in the insoluble, non-microbial, soil organic matter N pool in the top 5 cm. Our data suggest a shoot tissue turnover time of 12–20 yr for the evergreens and 3–5 yr for the deciduous shrubs. The overall ratio of vascular shoot to root tissue in the understory vegetation was ~1:2 (Table 2).

Species effects on ecosystem N cycling

^{15}N label partitioning between plant types.—Total plant ^{15}N label accumulation differed significantly between plant types and between sampling months (Fig. 8; plant type, $F_{5, 125} = 15.47$, $P < 0.001$; month, $F_{4,20} = 6.90$, $P = 0.001$; interaction, $F_{20, 125} = 1.78$, $P = 0.030$, $LSD_{\text{within months}} = 0.54$). In particular, vascular plant N uptake in October was dominated by the evergreen *E. hermaphroditum* (Fig. 8), corresponding with its large biomass above and below ground (Table 2). By contrast, ^{15}N label uptake by the deciduous species *V. uliginosum* in October was low (Fig. 8), despite its relatively large biomass above and below ground (Table 2), resulting in lower concentrations of ^{15}N label compared to *E. hermaphroditum* (Fig. 8). This suggests that N uptake by *V. uliginosum* may have been physiologically restricted at the end of the growing season.

After the initial rapid ^{15}N uptake following labeling in mid-October, plant label accumulation ceased for all vascular species through the subsequent winter (Fig. 8), and then significantly increased after the spring thaw in late May (Fig. 8). There were strong differences in species capacity for acquisition of the ^{15}N label in spring and early summer that were related to physiological uptake traits, rather than biomass differences. In particular, the relatively nonwoody species (*V. vitis-idaea*, *V. myrtillus*, and the herbs) acquired substantially more ^{15}N label per unit biomass (Fig. 8; plant type, $F_{5, 102} = 19.02$, $P < 0.001$; month, $F_{4,20} = 6.69$, $P = 0.001$; $LSD_{\text{within months}} = 5.46$), despite their relatively low biomass (above and below ground) compared to the other vascular species (Table 2).

Moss accumulation of ^{15}N label was characterized by substantial initial uptake in October that resulted in higher ^{15}N concentrations than in any of the vascular plant types (Fig. 8). Direct shoot contact with the freshly labeled soil surface may have favored ^{15}N accumulation in this period. Apparent subsequent changes in moss ^{15}N enhancement during winter and summer (Fig. 8) did not result in any significant differences in tissue ^{15}N label concentration (Fig. 8), suggesting that

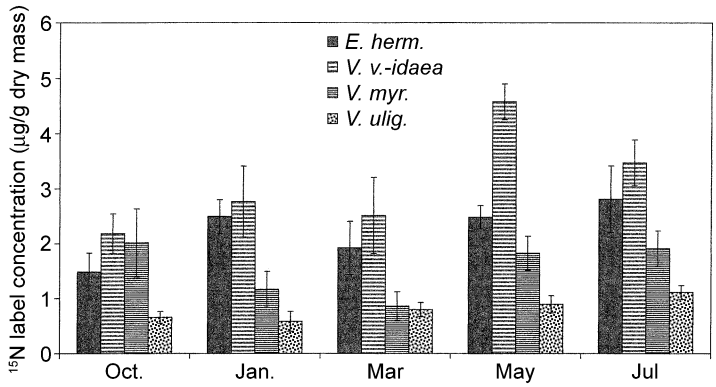

FIG. 9. Species differences in coarse root ^{15}N label tissue concentration in winter and summer (mean ± 1 SE). Herbs have not been included because data were only available for the July harvest.

they were most likely due to random differences in sampled biomass between months (Fig. 7), rather than to seasonal changes in moss ^{15}N uptake. Hence, although mosses acquired an appreciable proportion of total plant ^{15}N during the initial labeling phase in early winter, they did not influence subsequent ecosystem ^{15}N cycling within the time frame of the experiment.

Internal allocation of ^{15}N label within vascular plants.—Coarse root ^{15}N tissue concentrations varied significantly between species and between sampling months (Fig. 9; non-herb species, $F_{3,60} = 48.46$, $P < 0.001$; month, $F_{4,20} = 6.40$, $P = 0.002$; interaction, $F_{12,60} = 2.26$, $P = 0.020$; $LSD_{within\ months} = 0.90$). In general, the evergreen species tended to have significantly higher coarse root ^{15}N concentrations than did deciduous species at most sampling times (Fig. 9). Fine root tissue ^{15}N concentrations were substantially greater (~5–15 μg ^{15}N/g dry mass) than coarse root concentrations (Fig. 9), but did not vary significantly between species or sampling month (data are available only for October and July, and are not shown). These results demonstrate a pattern of rapid ^{15}N uptake by fine roots immediately after labeling at the onset of winter, followed by accumulation in coarse roots.

Species differed significantly in ^{15}N enhancement pool sizes of both old and new shoot tissues. ^{15}N enhancement in old shoots was generally highest in the evergreen *E. hermaphroditum* (Fig. 10; non-herb species, $F_{3,74} = 4.42$, $P = 0.007$; $LSD_{within\ months} = 0.10$), corresponding with its substantially greater aboveground biomass (Table 2). By contrast, ^{15}N enhancement in old shoots of *V. uliginosum* was significantly lower than in *E. hermaphroditum* in July (despite its relatively high biomass; Table 2). New shoot ^{15}N enhancement in July differed significantly between species (Fig. 10, vascular species in July only, $F_{4,20} = 2.81$, $P = 0.05$; LSD = 0.29). In particular, the deciduous shrub *V. myrtillus* acquired significantly more label than the other vascular species, corresponding with its relatively high shoot production rates (Table 2). Nevertheless, there was also a significant species effect on concentrations of ^{15}N in new shoot tissue (vascular species in July only, $F_{4,11} = 7.59$, $P = 0.003$; LSD = 10.46; data are not shown) indicating strong differences in species capacity to load actively growing tips with recently acquired N. Together, these results indicate significant species effects on ^{15}N enhancement within new and old shoots that were due to species differences in physiological capacity to translocate label within shoots, as well as inherent differences in shoot production.

Our results suggest that differences in species capacity to resume uptake and redirect allocation may be strongly related to differences in species productivity. Those plant types with highest aboveground production per unit biomass (i.e., fastest tissue turnover times) and highest aboveground production per unit total plant N (i.e., herbs, *V. myrtillus*; Table 2) allocated significantly more of their acquired ^{15}N label to new shoot tissue (Fig. 11; species, $F_{4,11} = 20.37$, $P < 0.001$; LSD = 2.6%). Together, our results suggest: (1) that overall productivity in the understory vegetation is strongly dependent on seasonal effects on N availability; and (2) that species contributions to ecosystem productivity depend on differences in species physiological capacities for N uptake and tissue allocation as well as relative biomass.

DISCUSSION

The influence of plants on ecosystem N cycling

Our experimental results demonstrate that intact plants had a negligible effect on soil microbial growth or microbial N accumulation in this ecosystem over the sampling intervals of 6–12 wk. The treatment reduced ''fresh'' (i.e., recently fixed) C inputs from plants by removing all shoot tissue and a large proportion of the coarse root material, as well as preventing further C inputs to the soil system via leaf litter. We expect that the litter C input associated with decay of the *detached* roots in treatment plots would have been greatest in the summer prior to initial labeling and first harvest (Grogan and Chapin 2000). This interpretation is supported by the seasonal pattern and magnitude of fine root production (control plot data Fig. 2, Table 2), which together indicate almost complete turnover of

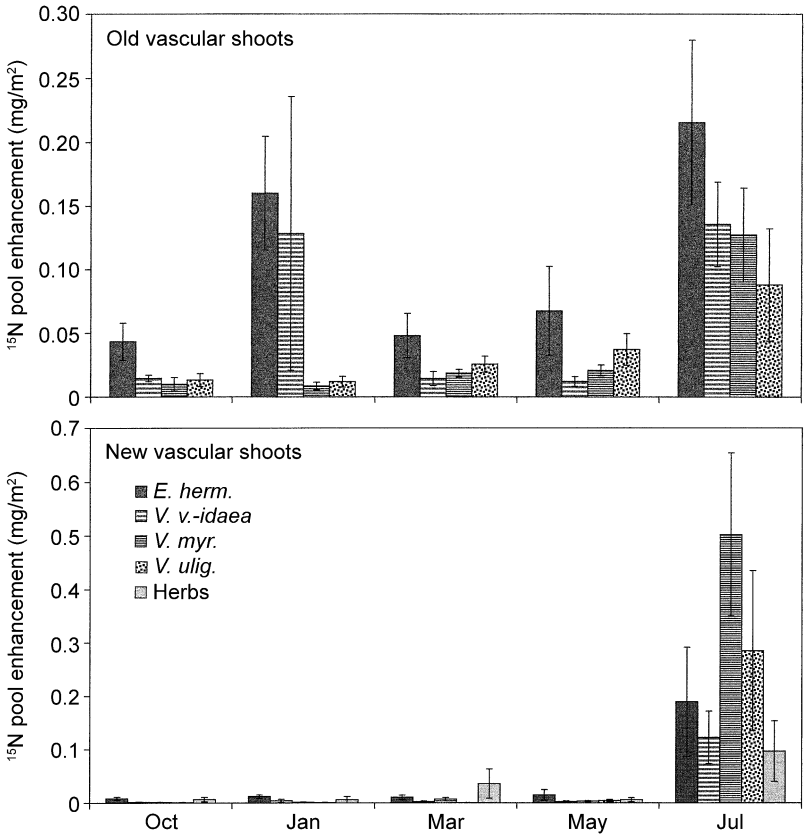

FIG. 10. Species differences in ^{15}N label pool sizes in old and new vascular shoots (upper and lower graphs, respectively) in winter and summer (mean ± 1 SE).

fine roots within a single growing season. Furthermore, although litter C inputs associated with turnover of the *living* fine roots present in treatment plots throughout the experiment (Fig. 2) may have been substantial, they are unlikely to exceed those in control plots. Nevertheless, the increase in fine root biomass of treatment plots during summer, its ongoing ^{15}N accumulation throughout the experiment (~30% of control; data are not shown), and the presence of some supporting coarse root biomass (Fig. 2) all suggest that at least a portion of these fine roots were actively growing, and therefore exuding C. However, relative to control plots, their detachment from photosynthetic C source tissue is likely to have severely diminished the ongoing supply of plant C to the rhizosphere through the experiment. Accordingly, we interpret the absence of treatment effects on microbial pools as indicating that microbial growth and N accumulation were largely independent of ''fresh'' C inputs associated with plants, at least within the first year of treatment (Fig. 3).

The sheer magnitude of the MBN pool compared to the soil solution pool and its rapid turnover compared

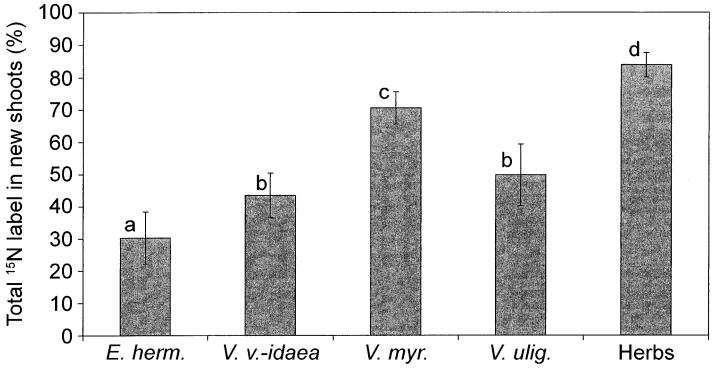

FIG. 11. Species differences in allocation of acquired ^{15}N label to new shoot tissue in July (mean ± 1 SE). Columns identified with different superscript letters are significantly different ($P < 0.05$).

to plants indicate that MBN dynamics dominate annual ecosystem N fluxes here (Figs. 3 and 4) and in other high-latitude ecosystems (Jonasson et al. 1999). In our case, we detected no significant changes in the MBN pool due to the presence of intact plants or between sampling months (Fig. 3). However, the use of an isotopic label allowed us to demonstrate that a substantial amount of recently acquired N (i.e., ^{15}N label) was released from the microbial pool during the winter–summer transition (Fig. 5). The correspondence of a significant drop in $MB^{15}N$ with a significant rise in plant ^{15}N during the winter–summer transition strongly suggests that plants could rapidly and directly acquire some of the N released by soil microbes during this period (Fig. 6). Furthermore, our results demonstrate that plants had the capacity to rapidly take up available inorganic N at the onset of winter, suggesting that plants could also have the potential to acquire N that might be released from microbes during the onset of freezing in early winter. Nevertheless, the absence of any significant effect of intact plants on MBN or $MB^{15}N$ pools in either winter or summer suggests that plant competition with microbes for available N was negligible from a microbial perspective. Thus, although plants clearly have substantial influences on microbial N cycling over time scales from hours to months (e.g., by competing for N uptake and providing root exudates), and over time scales greater than years (e.g., litter production and tissue chemistry), our results suggest that the annual pattern of microbial N-cycling activities was relatively independent of plant influences.

Ongoing microbial N mineralization activity beneath snow cover was indicated by significantly increasing soil ammonium concentrations during winter (Fig. 4). The relatively high NH_4^+ concentrations in treatment plots in October suggest that the removal of plants promoted accumulation of inorganic N during the summer preceding labeling, perhaps due to the elimination of plant N uptake, or enhanced N mineralization due to more favorable environmental conditions. Our results clearly demonstrate that plants were able to take up substantial ^{15}N during the October labeling period, but not later in the winter (Fig. 6). The absence of significant plant ^{15}N enhancement during the remainder of winter suggests that the increasing soil NH_4^+ concentrations in control plots through the winter may have been due to the cessation of plant uptake despite continuing microbial mineralization. Together, these results indicate that direct plant influences on N cycling in this ecosystem were confined to the period from late spring to early winter, whereas microbial activity continued throughout the year.

Seasonal controls on microbial and plant N accumulation

To our knowledge, this is the first ^{15}N labeling study to specifically investigate wintertime N-cycling activity and partitioning between microbes and plants in a high-latitude ecosystem. Through the added sensitivity provided by isotope labeling, our results strongly suggest that the winter–summer transition is a critical event for annual N cycling. In alpine snow-covered tundra, gradual increases in MBN over winter have been observed up until snow melt, followed by rapid fluctuations over the weeks immediately thereafter (Brooks et al. 1998). Our data from samplings over a longer time interval on either side of spring thaw indicate that MBN on an annual time scale is well buffered against seasonal effects (Fig. 3). However, the ^{15}N enhancement data for $MB^{15}N$ and $DT^{15}N$ pools indicated some variations during winter and significant declines at the end of winter (Fig. 5) that were coincident with a significant increase in plant ^{15}N accumulation. The ^{15}N enhancement of the DTN pool constituted $\sim$0.1% of total N in that pool, and $\sim$0.01% of the total N in the microbial biomass; ^{15}N enhancement of the microbial pool constituted $\sim$0.025% of the total N in the microbial biomass. Our results indicate that ^{15}N isotopic enhancement levels (rather than changes in total N pools) are a much more sensitive way of detecting significant effects on turnover and N transfers in/out of these relatively large N pools. Thus, although we observed no *net* release of N from the microbial pool through the experiment (Fig. 3), the added sensitivity provided by an isotopic label has allowed us to detect a period of rapid N turnover within the microbial pool that is of major significance for the functioning of this ecosystem.

The results provide strong evidence to support the hypothesis that arctic and alpine N cycling is characterized by a strong release of N from microbes at spring thaw, part of which is acquired by plants during the following summer (Lipson et al. 1999). Our data are confined to the period from early October to mid-July. The relative importance of the freeze–thaw period as opposed to the subsequent early-summer period in mobilizing ^{15}N from the microbial pool, and the impact of the late-summer period (after the mid-July harvest) on the annual N cycle were not determined. However, several other year-round field studies indicate that annual patterns of N supply in seasonally snow-covered soils are dominated by a major increase in soluble N pools at spring thaw due to the combination of sharp temperature fluctuations and saturating soil conditions (Dowding et al. 1981, Schimel et al. 1996, Lipson et al. 1999). Furthermore, summertime net N mineralization rates in tundra tend to be minimal (Giblin et al. 1991, Nadelhoffer et al. 1992, Schmidt et al. 1998). Therefore, our study indicating substantial microbial ^{15}N release and corresponding plant ^{15}N uptake during the transition from winter to summer strongly suggests that the principal control on the annual pattern of transfers (and potential losses) in this ecosystem is the seasonal environmental change associated with spring thaw.

Our microbial biomass carbon results are similar to those of previous studies in alpine tundra that indicated rising MBC during the winter months, followed by a sharp reduction during the transition from frozen winter soils to thawed soils in spring–summer (Lipson et al. 1999). A recent set of soil incubation experiments concluded that the decline in microbial biomass after snow melt may be controlled by a combination of decreases in soil carbon availability and increases in temperature (Lipson et al. 2000). Our results indicating a strong decrease in MBC between May and mid-July suggest that any decline in MBC due to limiting C availability cannot be directly associated with the presence of living plants or litter produced in the previous year. We conclude that the common pattern of declining MBC during the winter–summer transition is most likely determined by seasonal environmental factors rather than plant-associated effects.

Our study provides several important insights into ecosystem N cycling in the Arctic. First, the seasonal pattern of MB^{15}N pool data can be used to estimate microbial N turnover rates. The label was added in inorganic form in the previous October, and may not have been uniformly incorporated within all cellular components of the microbial biomass by the May or July harvests. Thus, the strong release of isotope during the winter–summer transition may be most representative of the labile (cytoplasmic) N fraction within the microbial biomass. If we assume uniform label incorporation, slightly greater than half of all label within the MB^{15}N pool was released by mid-July in the growing season following winter labeling, suggesting a turnover period of ~2 yr. However, although there is substantial evidence to suggest that the particular environmental conditions associated with spring thaw are the principal driver of MBN turnover on an annual cycle, the contribution of ongoing turnover between the mid-July harvest and the onset of winter was not determined in this study. Therefore, assuming uniform label incorporation, we conclude that the observed rate of ^{15}N release indicates a turnover time for N in the soil microbial pool of 1–2 years. Second, our results raise questions as to the efficiency of internal transfers of mobilized N within the ecosystem during the growing season. Understory vascular plants acquired the equivalent of 14% of the ^{15}N label released from the microbial ^{15}N pool between May and July (Fig. 6). Furthermore, total ecosystem ^{15}N retention was significantly reduced over this same period in both control and treatment plots. Thus, a substantial amount of label was unaccounted for in the final harvest, suggesting that it had either been lost from the ecosystem (as leachates or gases), or transferred to deeper soil horizons. Total annual N inputs via atmospheric deposition in this locality are ~0.06 g N/m^2 (Kindbom et al. 1997), and biological N fixation in high-latitude ecosystems rarely exceeds 0.18 g N/m^2 (Dowding et al. 1981). Assuming that N cycling is in equilibrium in the complete ecosystem, annual N losses are likely to be of an equal magnitude. Thus, total N loss rates are small relative to our estimate of MBN turnover (2.5–5 g N·m^{-2}·yr^{-1}), indicating that the most likely explanation for the substantial amount of unaccounted ^{15}N label in the July harvest is not that it represents an ecosystem loss, but that it had moved down the soil profile and would normally have been accessed by birch roots (had they not been precluded from the study). If so, these data suggest that annual N cycling in this ecosystem is characterized by highly efficient internal N transfers between microbes and plants, with only minimal external inputs and losses. To investigate the longer term fate of the added label within understory ecosystem components, as well as total losses, the experiment has been designed so that additional harvests within these plots can be conducted in the future.

Plant species differences in N accumulation

This study demonstrates that substantial understory plant N uptake can occur in subarctic ecosystems during the onset of winter as well as during the summer months. After the initial uptake period and a long dormant overwinter phase in which negligible uptake or translocation occurred, there was a rapid resumption of ^{15}N label uptake in early summer (Fig. 6) and a strong internal diversion of label toward aboveground rather than belowground tissue (Fig. 7). This general seasonal pattern masked significant species differences in uptake and translocation capacities that depended on differences in physiological uptake capacity between species, as well as relative biomass. Early-winter uptake was dominated by *Empetrum hermaphroditum*, the dominant biomass species, whereas uptake by *Vaccinium uliginosum* was particularly low, despite its relatively large biomass. Furthermore, total vascular plant ^{15}N enhancement in July (Fig. 8) and ^{15}N enhancement in aboveground parts (Fig. 10) were strongly influenced by differences between species in physiological uptake capacity for available ^{15}N. Herbs, *Vaccinium vitis-idaea* (evergreen), and *V. myrtillus* (deciduous) all accumulated substantially more added ^{15}N per unit biomass (i.e., higher tissue ^{15}N concentrations) than did *E. hermaphroditum* (evergreen) and *V. uliginosum* (deciduous) (Fig. 8). The latter species are characterized by substantial wood tissue production during secondary thickening growth of above- and belowground stems. Thus, N uptake capacity in early summer did not appear to be related to differences in leaf longevity traits, but rather to species differences in the extent of wood development. Species with slender belowground stems may have a relatively dynamic root system that can respond more rapidly to flushes in soil nutrient availability. In addition, species could have varied in ^{15}N uptake capacity because of differing reliances on organic and inorganic N forms, as well as differing degrees of ericoidal mycorrhizal association (in the shrubs). Our data indicate significant species differ-

ences in internal allocation of newly acquired N to new and old shoot tissues that were not consistently related to differences in relative biomass (Fig. 10, Table 2). Finally, in terms of overall allocation of acquired label, the herbs and *V. myrtillus* were able to direct proportionally more of the label to active growing points of the shoot (Fig. 11). These results, together with the strong correlations between ^{15}N allocation capacity and estimated aboveground productivity (Table 2), suggest that species differences in productivity in this ecosystem are related to plant physiological differences in N uptake capacities and internal translocation that may be a consequence of differences in the extent of wood development.

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