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Nitrogen Uptake During One Year in Subarctic Plant Functional Groups and in Microbes After Long-Term Warming and Fertilization

Pernille L. Sorensen,* Anders Michelsen, and Sven Jonasson

Terrestrial Ecology Section, Department of Biology, University of Copenhagen, Oester Farimagsgade 2D, 1353 Copenhagen K, Denmark

Abstract

For the first time in an arctic long-term warming and fertilization experiment, the short-term (days) and longer-term (month and year) nitrogen (N) uptake and allocation in plants, microbes, and soil pools were studied, with ^{15}N-labeling of an organic nitrogen form, glycine. The long-term warming and fertilization had no marked effect on soil inorganic N content, but both dissolved organic N (DON) and plant biomass did increase after fertilization. Soil microbes initially immobilized most of the added ^{15}N, but in the following months, they lost two-thirds, while label concentration in plants increased. After a year, however, the ^{15}N recovered in microbes was still 10-fold higher than that in the plant biomass, showing the high importance of soil microbes in nutrient retention in arctic ecosystems, irrespective of the impact of long-term warming or fertilization. The effects of the treatments on the uptake of label by deciduous shrubs and evergreens paralleled that of their N pool sizes, suggesting that their N uptake potential was unaffected by long-term warming and fertilizer addition. Mosses and herbs had high uptake potential but in fertilized plots they took up less ^{15}N, that is, they were N saturated. The fraction of ^{15}N in microbes tended to decrease after fertilization, but this was an effect of higher N pool dilution after 1 month and a year, and not due to lower initial uptake. Although the concentration of soil inorganic N did not change after fertilization, both increased DON and the results of the ^{15}N label addition showed that the N availability in the ecosystem had increased. By contrast, warming had little effect on soil N pools and microbial ^{15}N uptake, and, hence, had no detectable effects on ^{15}N accumulation.

Key words: climate change; fertilization; microbial immobilization; plant N uptake; warming.

Introduction

Plant growth in most arctic ecosystems is limited by low nitrogen (N) availability (Chapin and Shaver 1996; Jonasson and Shaver 1999; Jonasson and others 1999a). Microbial mineralization of soil organic matter (SOM) is also commonly limited by nutrient deficiency (Weintraub and Schimel 2003; Schimel and others 2004; Mack and others 2004) in addition to low temperatures (Post and others 1982) and high soil moisture (Flanagan and Veum 1974). In these nutrient limited arctic ecosystems, microbes retain almost all gross mineralized N (Jonasson and others 1993; Schmidt and others

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*Corresponding author; e-mail: pernills@bi.ku.dk

1999), leaving little to the pool available for plant uptake.

The microbes efficiently recycle the sequestered N, leading to low net N mineralization rates and turn-over times of N in the microbial biomass of several years (Jonasson and others 1999a). Net N mineralization rates may commonly be underestimated, because the rates usually have been measured with the buried bag technique without the presence of plants (Eno 1960). This technique does not account for probable mobilization of ''extra'' N by plants if they are present during the mineralization process (Jonasson and others 2006).

Increase of air temperature by 3–5°C in the Arctic over the course of this century as a consequence of anthropogenic increase of greenhouse gasses (IPPC 2001; ACIA 2005) is likely to increase the rates of nutrient cycling and decomposition (Nadelhoffer and others 1997; Jonasson and others 1999b). Thus, by increasing mineralization rates, warming probably will result in loss of organic C from the soil organic matter accompanied by release of plant available nitrogen (Nadelhoffer and others 1992; Mack and others 2004).

At the site of this experiment, a dry heath near Abisko, Northern Sweden, more than a decade of climate manipulations has, however, led to only moderate ecosystem responses. Warming has increased mineralization rates insignificantly, and plant and soil microbial biomass also show relatively small changes (Jonasson and others 1999b; Ruess and others 1999; Schmidt and others 2002). Heavy fertilization, by contrast, has increased plant biomass substantially and has changed plant community composition after a few years (Graglia and others 2001), whereas changes in microbial biomass and microbial community composition have been moderate and only appeared after manipulations for more than a decade (Rinnan and others 2007).

Here, we added an isotopically rare N source (^{15}N) to the ecosystem with subsequent measurements of ^{15}N distribution in plants, microbes, soil, and soil solution after a few days, 1 month, and 1 year. The overall objective was to reveal short- (days) and longer-term (month and year) allocation patterns of the added ^{15}N and to examine if the allocation of N changed as a consequence of long-term (13–14 years) warming and fertilization.

The ^{15}N label addition had three main detailed objectives: (i) To test the ability of plants to access added N in competition with soil microbes. Short-term labeling studies of hours to days have indicated that microbes in the short-term monopolize nearly all added ^{15}N in nutrient limited ecosystems. In longer term (weeks to months) plants may acquire more of the added ^{15}N while the fraction of the ^{15}N recovered in microbes may decrease. However, data on the long-term fate of added N are scarce. Therefore, this study followed the added ^{15}N for a whole year to examine the longer-term distribution of added ^{15}N between plants, microorganisms, and soil N pools. (ii) To use the ^{15}N label addition to estimate turn-over rates of microbial N in situ to give a more realistic result than when using the buried-bag method, by which plant roots are excluded. (iii) To provide information on ecosystem nutrient status after long-term warming and fertilization, as the rate of plant N uptake changes according to the plant demand for N. Under nutrient limitation, it has been suggested that plants will take up N with high rates until their growth demand for N is met (Bassirirad 2000). Plants that are well supplied with N will, in contrast, show reduced uptake rates compared to N limited plants (Jones and others 1994; Persson and Näsholm 2003).

Methods

Site Description and Experimental Design

The experiment took place near Abisko in northern Swedish Lapland, in a subalpine heath just above the forest line at 450 m above sea level. The bedrock consists of base-rich mica shists, and the vegetation is dominated by the evergreen dwarf-shrub *Cassiope tetragona*. The organic soil at the site is 12–15 cm deep and has a pH of about 7.0. The climate is montane subarctic, with a growing season of approximately 3 months lasting from mid-June to early-mid-September; see Havström and others (1993) and Michelsen and others (1996a) for more detailed accounts on climate and vegetation.

The experimental setup of the site was initiated in 1989. It consisted of four treatments: control, warming, fertilizer addition, and warming + fertilizer addition. The treatments were replicated across six blocks within an area of approximately 800 m^2. Each year in early June, just after snowmelt, and until the end of August or early September, the temperature was raised by erecting dome-shaped plastic greenhouses with a 1.2×1.2 m^2 surface area. The greenhouses enhanced the air temperature by 3.9°C. The soil temperature was enhanced by 1.2–2.0°C above an average temperature of 7.7°C in the control plots, and the greenhouses reduced photosynthetically active radiation by 9% (Havström and others 1993; Michelsen and others

1996a; Rinnan and others 2007). Fertilizer was added each year soon after snowmelt in June at a rate of 4.9 g m^{-2} for N and 1.3 g m^{-2} for P in 1989. From 1990 to 2000, the additions were 10.0 and 2.6 g m^{-2}, respectively. No fertilizer was applied in 1993, 1998, and 2001.

The label addition took place on 24 June 2001 as a pulse labeling to the soil with ^{15}N-glycine (98%, Cambridge Isotope Laboratory). The glycine was injected within 20 × 20 cm^2 plots with a syringe guided by a grid frame with 25 holes and with each point receiving 10 ml resulting in a total of 0.128 g N added per m^2. The label was evenly distributed to the soil column from just below the green moss mat to a depth of approximately 5 cm.

Sampling and Analyses of Plants and Soil

On 25 July, 27 July, and 24 August 2001, and on 21 July 2002, that is, 1 day, 3 days, 1 month, and 1 year after label addition, two soil cores of 4 cm diameter were collected from the organic horizon to a depth of 6 cm in each plot. Soil from the two cores was pooled to one soil sample per plot and roots were sorted out and kept. Ten grams of the sorted, fresh soil was fumigated with $CHCl_3$ for 24 h to release the N in the soil microbial biomass, after which the soil was extracted for 1 h in 50 ml 0.4 M K_2SO_4 (Brooks and others 1985). The extracts were filtered through Whatman GF-D filters and frozen until analyses. Another 10 g fresh soil was treated as above, but without the $CHCl_3$ fumigation to recover soil inorganic N. Parts of the extracts were analyzed for NH_4^+–N by the indophenol method and for NO_3^-–N by the cadmium reduction method (Allen 1989). The NO_3^- content generally was below the detection limit of 0.025 μg g^{-1} SOM and will not be reported. To obtain microbial N, 2.5 ml of fumigated and unfumigated K_2SO_4 extract was digested with 1.25 ml concentrated H_2SO_4 with 4.7 mg H_2SeO_3 and 0.25 ml 30% H_2O_2 added, after which H_2O was added to a volume of 25 ml. The samples were digested at 400°C for 1 hour. The N content was analyzed by the indophenol method. Microbial N was calculated by subtracting the N in digested, unfumigated extracts from that in digested, fumigated extracts. The microbial N content was calculated assuming an extractability of 0.4 (Schmidt and others 2002).

Amino acid N concentrations were analyzed in water extracts of soil sampled in the control plots using 10 g soil extracted for 1 h in 50 ml demineralized water and subsequently filtered. The amino acid concentration in the extract was measured using an ion chromatography system from Dionex, equipped with electrochemical detection with a gold amperometry cell and the analytical column AminoPac PA10 with separation of arginine, alanine, aspargine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, valine, tryptophan, and tyrosine. Their concentrations were summed to a measure of total soil amino acid N concentration.

At the same time as the soil was sampled in 2001, we collected five randomly chosen shoots of each of the plant functional groups: mosses, deciduous shrubs, evergreen shrubs, herbs, and the dominant evergreen species *Cassiope tetragona*. On 21 July 2002, that is, 1 year after labeling, all aboveground vegetation was harvested, brought to the laboratory, and sorted into groups as above for determination of biomass. Deciduous and evergreen shrubs were divided further into leaves and stems. All plant material was washed several times with 0.5 mM $CaCl_2$ to remove any adhering label. Ten grams of fresh soil remaining after sorting was dried at 70°C for determination of soil moisture content. All dried plant and soil samples were milled and brought to Copenhagen where they were kept dry and dark until chemical analyses. At the final harvest, the 6–12 cm soil horizon was additionally sampled for total soil ^{15}N analysis only.

The approximately 1,350 plant and soil samples were packed in tin capsules, each containing about 5 mg material, and their $^{15}N/^{14}N$ isotope ratio and the total nitrogen content were analyzed with an Isoprime isotope ratio mass spectrometer coupled to an Eurovector CN analyzer (Micromass-GV Instruments 2002). Values for natural abundances of ^{15}N for plants and soil were obtained earlier at the same site (Michelsen and others 1996b).

The microbial ^{15}N content was determined by acid-trap diffusion (Stark and Hart 1996). Four milliliters of 12.5 M NaOH was added to flasks containing 20 ml digested fumigated or unfumigated extract to raise the pH to above 13 and thereby convert the NH_4^+ to NH_3. KCl was added to increase the ionic strength of the solution before sealing the flasks. The NH_3 diffused to an acidified (15 μl 1.5 M H_2SO_4) Milipore quartz filter (0.8 cm diameter) suspended over the solution while gently shaking the flasks at 75 rpm. After 5 days, the filters were removed and dried before analyses for $^{15}N/^{14}N$ isotope ratios as described above. To calculate the microbial ^{15}N recovery, we used the microbial N pools measured with the indophenol method, and the ^{15}N atom percentage enrichment

measured after the acid-trap diffusion, as standards of known atom percentage ^{15}N included throughout the procedure showed variation between 75 and 100% recovery of N, but very high precision of $\delta^{15}N$-values. $\delta^{15}N$ in dissolved total N (DTN), that is, the sum of dissolved organic and inorganic N, was determined from the digested, unfumigated extracts.

Atom percentage enrichment for each of the plant, soil, and microbial components was determined by subtracting the natural ^{15}N abundance of the control samples from the atom percentage of labeled samples. To calculate ^{15}N recovery of DTN, microorganisms, and plants, we multiplied ^{15}N atom percentage enrichment of the pool by the pool size and divided this value by the amount of label added, that is, [atom% $^{15}N_{labeled}$ − atom% $^{15}N_{non\text{-}labeled}$] × N pool size/total added ^{15}N. For aboveground plant ^{15}N recovery, we used the biomass data obtained after the 2002 harvest.

Statistical Analyses

Data means were compared by analysis of variance (ANOVA), with type III sums of squares by the GLM procedure, followed by Tukey's test to localize the significant differences (SAS Institute v8.02, 2001). Data with $P < 0.05$ were considered statistically significant, but also tendencies toward significance ($P < 0.1$) are reported. Data on soil pools and ^{15}N recovery were examined with repeated measures two-factor ANOVAs with warming and fertilization as main factors, and with interaction between warming and fertilization included. Effects of treatments on aboveground biomass and N pools were tested with two-factor ANOVAs with warming and fertilization as main factors, and with their interaction. A block effect was included in the statistical tests when the *P*-value of the block effect was below 0.1. Before the analyses, all data were tested for homogeneity of variances by Levene's test, and if necessary transformed appropriately.

Results

Soil Nitrogen Pools, Plant Biomass and Nitrogen Status

SOM, DON-N, and microbial N concentrations increased significantly with fertilization (Figure 1A, C, D) However, the soil concentration of ammonium did not change significantly with fertilization,

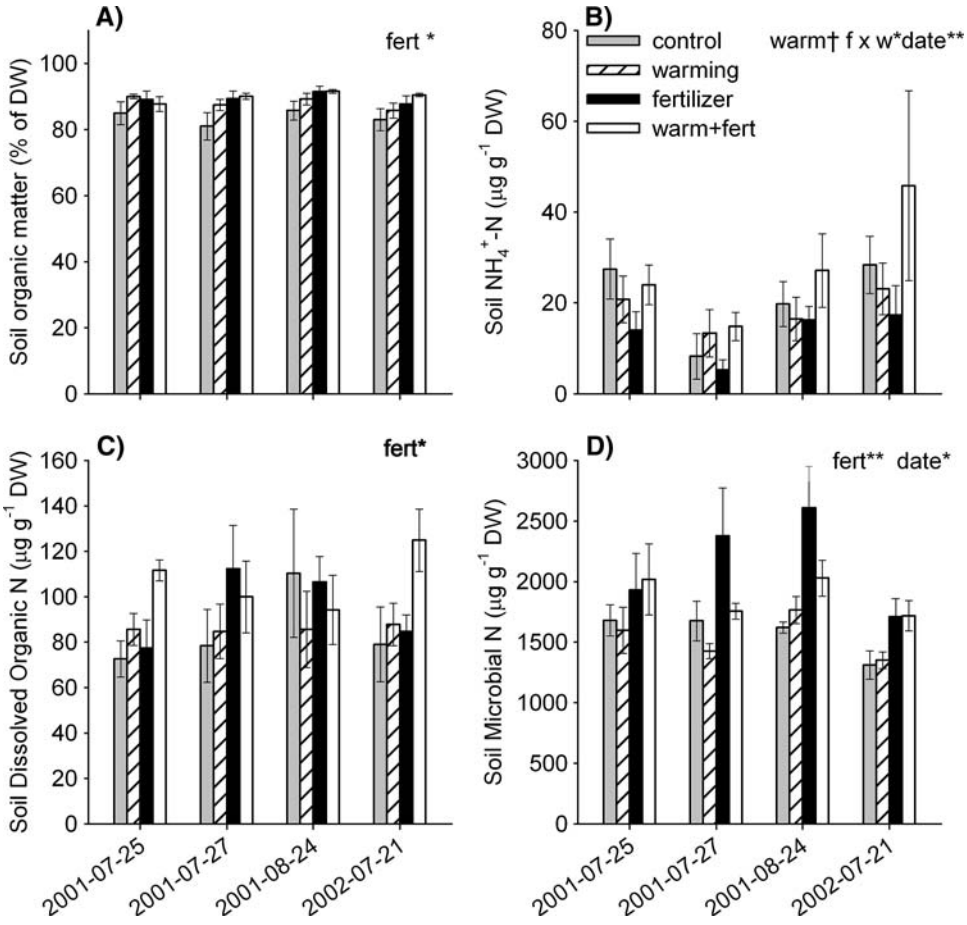


Figure 1. Seasonal and treatment effects on: **A** Soil organic matter, **B** Soil ammonium concentration, **C** Soil dissolved organic N, and **D** Soil microbial N concentration. (means ± SE, $n = 6$). The effects of warming and fertilization and their interactions were analyzed with repeated measures two-factor ANOVAs: † $P < 0.1$, *$P < 0.05$, ***$P < 0.001$.

neither when tested over the four sampling occasions (Figure 1B) nor when tested separately for the different harvest occasions (Tukey's test, $P > 0.33$). The amino acid-N concentration in the control plots was 1.55 ± 0.15 μg g^{-1} dw soil, of which the glycine content was 0.03 ± 0.01 μg g^{-1} dw soil. In the glycine treated plots, the ^{15}N-glycine immediately after labeling made up 90.9% of the soil amino acid-N and 99.8% of soil glycine-N concentration, corresponding to 18.1, 15.2, 16.5, and 12.1% of the total DON pool in the control, warmed, fertilized, and warmed + fertilized plots, respectively.

Total below- plus aboveground plant biomass increased after fertilization (Figure 2). The proportions among different plant functional types changed from dominance of *Cassiope* in control plots to higher proportions of herbs and deciduous shrubs in fertilized plots (Figure 2) with significant increases of the biomass of herbaceous plants ($P = 0.001$) and deciduous shrubs ($P = 0.002$). Also warming increased the biomass of deciduous shrubs ($P = 0.013$), but not the biomass of herbaceous plants. The moss biomass decreased in all treatments, mostly so in the ones with added fertilizer, and in the combined treatment the mosses almost vanished.

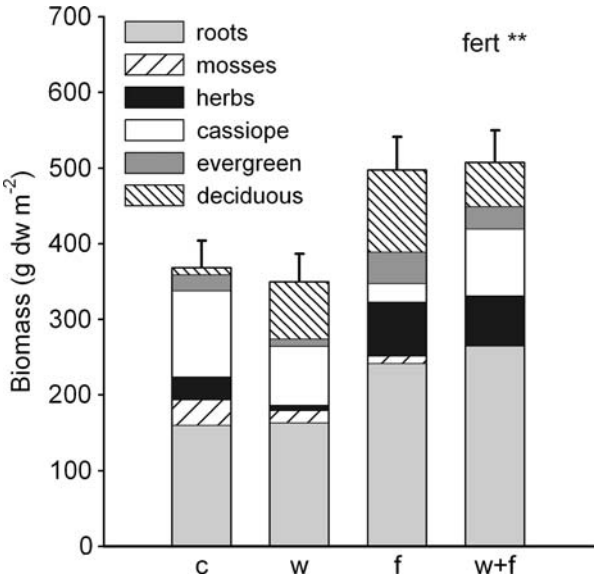


Figure 2. Effects of treatment on above- and belowground plant biomass (means + SE, $n = 6$). The root fraction is the overall mean of the four samplings and the aboveground biomass is from July 2002. The effects of warming and fertilization and their interactions were analyzed with two-factor ANOVAs: $^{**}P < 0.01$. *c*: control, *w*: warming, *f*: fertilization, *w* + *f*: warming plus fertilization.

Plant nitrogen concentration was affected significantly by fertilization in all plant functional types and tissue categories, except in herbs (Table 1). The N concentration increased significantly in fertilized plots in roots, mosses, *Cassiope*, evergreen shrubs, and in deciduous shrubs. The N pools therefore also increased or tended to increase by fertilization in all plant functional types, except in mosses (Figure 3). Because moss biomass decreased after long-term fertilization, the moss N pool diminished in the warmed + fertilized plots, but in the fertilized plots with higher moss biomass, the pools stayed at control levels (Figure 3B), even though the N concentration doubled (Table 1). The N pool of the herbs increased in fertilized plots (Figure 3C) because of increasing biomass (Figure 2), whereas the N concentration did not change significantly (Table 1). In contrast to fertilization, warming had fewer effects on the N concentrations and pools, except in mosses, which showed decreased N pools despite increased N concentrations due to reduction of their biomass, and in the deciduous plants, which showed increased N concentration and pool. However, the significant interaction between warming and fertilization (Table 1; Figures 2, 3G, H) indicate that the combined treatment decreased N concentration, N pool, and biomass in deciduous plants as compared to the effects of either the warming or fertilization treatments alone.

^{15}N Partitioning in Plants, Microbes and Soil Components

All plant functional groups took up N from the ^{15}N-glycine source, but with considerable variations among species and tissue types. The mean concentration of ^{15}N in roots integrated over the

Table 1. ANOVA Table for N Concentration Responses of Different Plant Fractions in July 2002 to Long-Term Warming and Fertilization

Plant fraction	*P*-values		
	Warming	Fertilization	Warm × Fert
Fine roots	0.7676	0.0008	0.7551
Mosses	0.0486	<0.0001	0.3590
Herbs	0.0846	0.1273	0.1812
Cassiope	0.1439	0.0083	0.5310
Evergreen leaves	0.9622	0.0450	0.8128
Evergreen stems	0.6344	0.0153	0.4135
Deciduous leaves	0.0035	0.0111	0.0165
Deciduous stems	0.0542	0.0040	0.2533

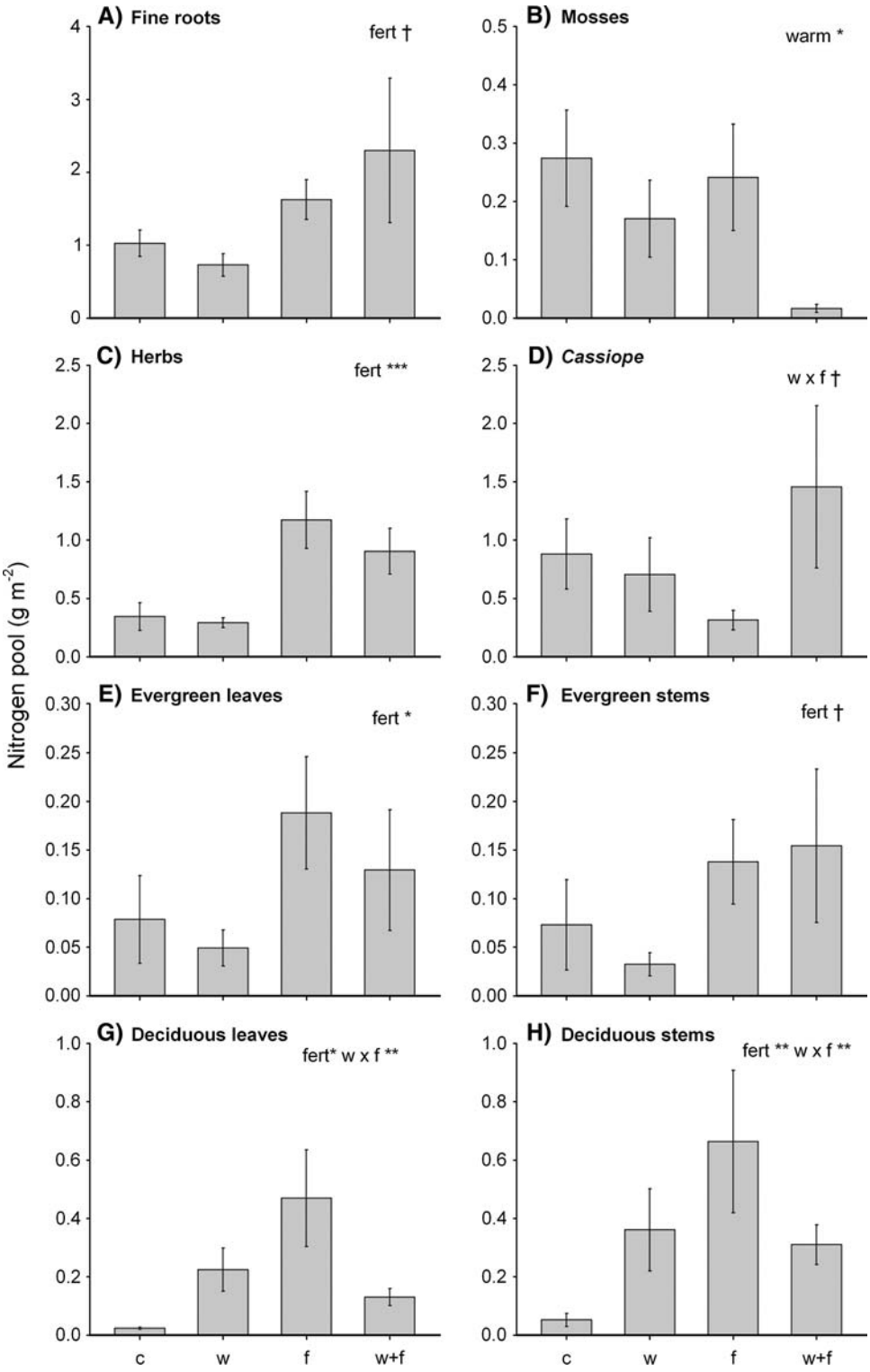


Figure 3. Treatment effects on nitrogen pools in July 2002 in: **A** Roots, **B** Mosses, **C** Herbs, **D** *Cassiope*, **E** Evergreen leaves, **F** Evergreen stems, **G** Deciduous leaves, **H** Deciduous stems (means ± SE, $n = 6$). The effects of warming and fertilization and their interactions were analyzed with two-factor ANOVAs: † $P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

different treatments 1 day after labeling was approximately 55 μmol g^{-1} N, and stayed at this level throughout the experiment.

The ^{15}N concentrations in fine roots were lower in fertilized than in unfertilized plots integrated over the entire experiment period ($P = 0.046$) (data on ^{15}N concentrations not shown). In spite of this, the ^{15}N recovery in fine roots tended to be higher in the fertilized than in the unfertilized plots (Figure 4A), because of the higher root biomass

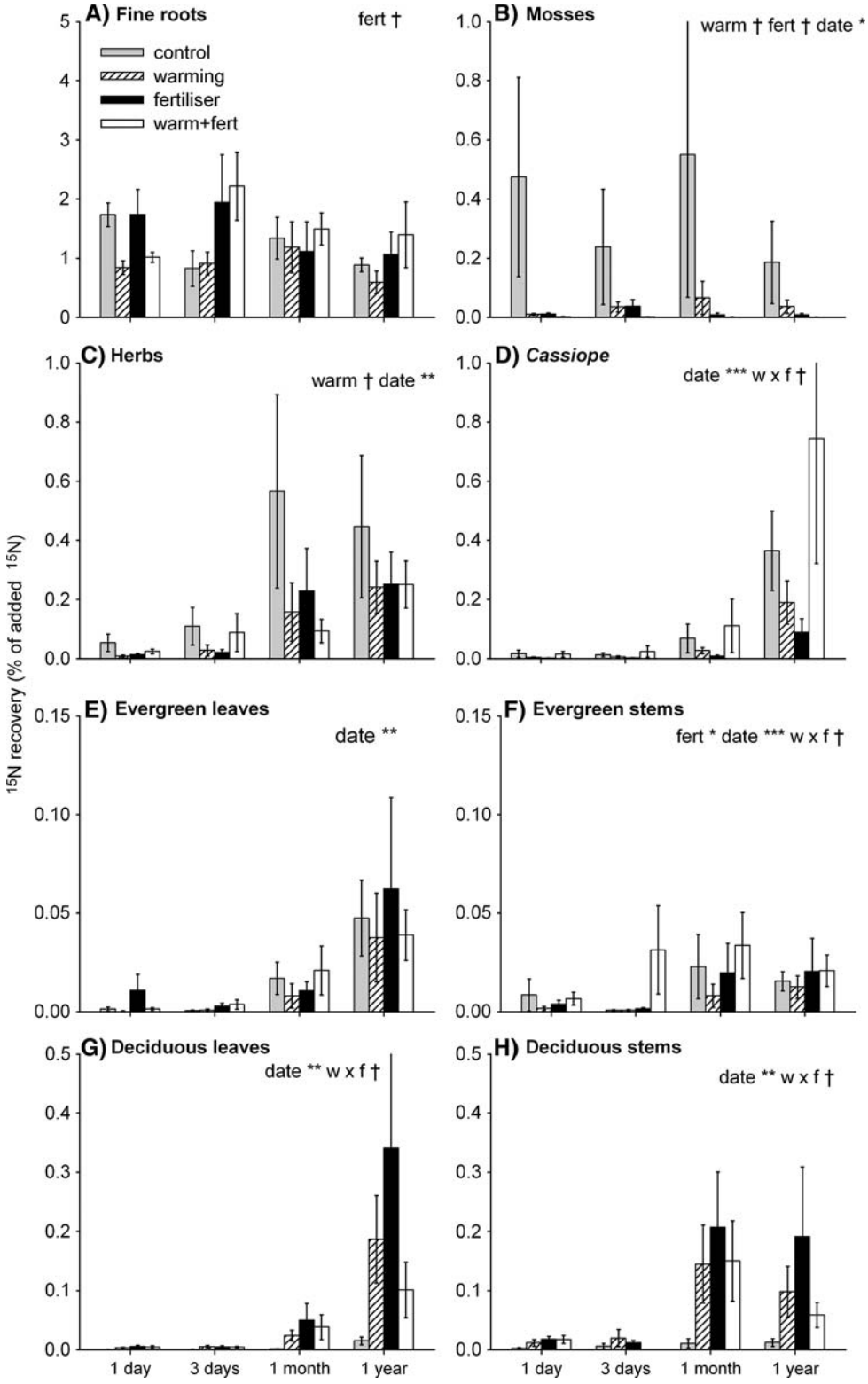
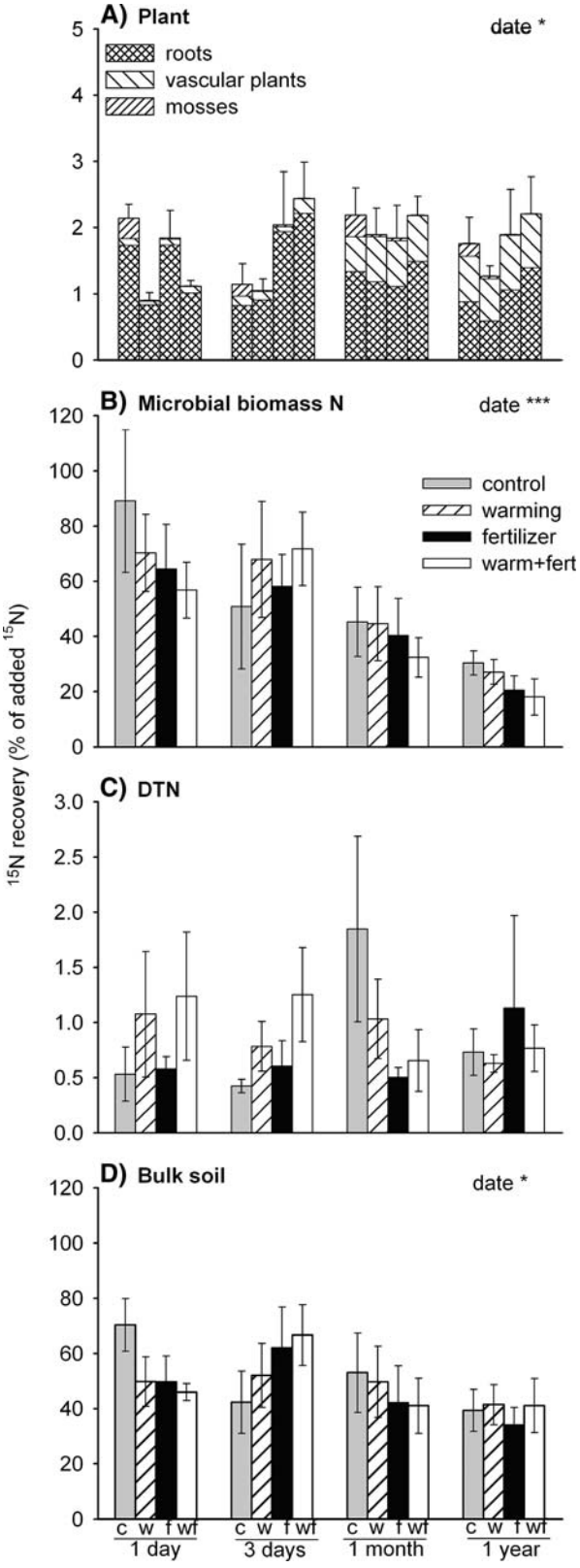


Figure 4. Seasonal and treatment effects on ^{15}N recovery (percent of added ^{15}N) in: **A** Roots, **B** Mosses, **C** Herbs, **D** *Cassiope*, **E** Evergreen leaves, **F** Evergreen stems, **G** Deciduous leaves, **H** Deciduous stems (means ± SE, $n = 6$). The effects of warming and fertilization and their interactions were analyzed with repeated measures two-factor ANOVAs: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

(Figure 2). The concentration of ^{15}N label in aboveground plant parts only responded to the treatments in mosses and herbs. Mosses had much higher label concentrations in the control plots than in the other plots and, consequently, also the moss ^{15}N recovery was high, although with large variation among replicates (Figure 4B). Herb ^{15}N concentration was significantly higher ($P = 0.008$) in unfertilized than in fertilized plots, reaching approximately 105 μmol g^{-1} N 1 month and 1 year



◀**Figure 5.** Seasonal and treatment effects on ^{15}N recovery in: **A** Plants, **B** Microbial biomass, **C** Dissolved total N, and **D** Bulk soil (means ± SE, $n = 6$). The effects of warming and fertilization and their interactions were analyzed with repeated measures two-factor ANOVAs: $^{*}P < 0.05$. *c*: control, *w*: warming, *f*: fertilization, *wf*: warming and fertilization.

after labeling, in contrast to concentrations of around 30 μmol g^{-1} N in the fertilized plots. The treatments only affected ^{15}N recovery significantly in evergreen stems (Figure 4F), whereas the other aboveground tissue types did not respond markedly (Figure 4C, D, E, G, H). However, as for the N pool, the ^{15}N recovery in deciduous plants in the combined treatment tended to be lower than in the single treatments, whereas an opposite trend of higher ^{15}N recovery in the combined treatment was observed in evergreens.

Label recovery changed significantly with time in all plant fractions except fine roots (Figure 4A). In all aboveground vascular plant components, the recovery increased strongly between 3 days and 1 month after the label addition and stayed at a similar level, or increased, the next 11 months (Figure 4C–H). The timing of ^{15}N accumulation in stems of the evergreen and deciduous species (Figure 4F, H) differed from the timing in the leaves (Figure 4E, G), as maximum values were attained already 1 month after labeling in the stems and did not increase further during the subsequent 11 months. The total recovery of ^{15}N in plants was initially about 1.5% of the added ^{15}N across treatments and increased to about 2% after 1 month (Figure 5A).

Label concentration in microbes was about 10-fold higher than in plants, reaching 4–600 μmol g^{-1} microbial N 1 day after labeling. There was a close to significant tendency ($P = 0.059$) for lower ^{15}N concentrations in microbes in fertilized plots. However, microbial ^{15}N recovery was not affected by the treatments (Figure 5B). The recovery decreased significantly with time from about 70% of the added ^{15}N 1 and 3 days after the label addition to about 30% recovery of added label after 1 year (Figure 5B).

One day after labeling, the recovery of ^{15}N in the soil DTN (DON plus NH_4^+) made up between 0.5 and 1.2% of the added label, and stayed at this low level during the entire experiment (Figure 5C). Integrated over the short-term harvests after 1 and 3 days, we found a significant increase in the ^{15}N-DTN concentration in warmed plots ($P = 0.035$), but integrated over the entire experiment period, there were no significant treatment effects (Figure 5C).

One day after ^{15}N label addition, the recovery in bulk soil was lower than in the microbes (Figure 5B, D), probably because we over-estimated initial ^{15}N uptake by microbes (see section ''Discussion''). We recovered 50–70% of added ^{15}N

in the bulk soil (0–6 cm depth) 1 day after label addition. In the 0–6 cm soil horizon, the recovery decreased significantly to 40% after 1 year (Figure 5D) whereas it was about 70% in the 0–12 cm soil horizon, indicating downward movement of label.

Discussion

Partitioning of Label Within Ecosystem Components; Microbial Dominance of N Cycling

Our data of high and rapid sequestration of added label agree with short-term sink dominance for added ^{15}N by microbes seen in other studies with measurements a few days after addition (Schimel and Chapin 1996; Grogan and Jonasson 2003; Nordin and others 2004). The subsequent strong decline in microbial ^{15}N from about 70% of the added label recovered a few days after addition to 30% recovered after 1 year indicates, as we expected, that part of the added label initially absorbed by the microbes was released within 1 year after sequestration. The release of more than half of the initially sequestered ^{15}N indicates that the microbial turnover time is less than 2 years and, hence, shorter than earlier reported from this ecosystem type (Jonasson and others 1999a). Also, Grogan and Jonasson (2003) found a microbial turnover time of about 2 years in a subarctic birch forest, based on estimates of turnover of ^{15}N. It is possible, however, that the recently absorbed microbial ^{15}N measured a few days after addition has been overestimated in relation to ^{15}N absorbed over a longer interval of time due to different degrees of metabolism and associated differences in extractability by chloroform (for example, Schimel and Chapin 1996). If so, the turnover time would be underestimated. This is, indeed, indicated by the recovery we measured in the microbes, which in some cases exceeded the recovery in the bulk soil, including the microbial fraction.

After 1 year, we recovered nearly the same amount of label in the plots as after a few days. The recovery in the DTN was unchanged, but the recovery in plants had increased, as we had expected, and, in addition, a major part was recovered in the bulk soil at greater soil depth than initially. The high recovery in deeper soil layers indicates a high transfer of N from microbes to the soil organic matter, which, indeed, has been reported previously in an Alaskan boreal forest (McFarland and others 2002) and in subarctic mesocosms sampled near the location of our experiment (Grogan and others 2004). The ^{15}N recovery in roots was stable throughout the experimental period, implying that the roots continuously allocated absorbed ^{15}N to the aboveground plant parts.

Among the plant groups, the ^{15}N concentration and recovery in herbs reached maximum levels already 1 month after labeling, at least in the unfertilized plots, and remained at the same level after 1 year, even though herbs lost the entire 2001 aboveground biomass before the last harvest in July 2002. Other labeling studies have also shown high allocation of recently acquired N to aboveground parts in graminoids and forbs (Schimel and Chapin 1996; Grogan and Jonasson 2003), showing that herbs give priority to productivity. The deciduous shrubs and the evergreens (including *Cassiope*), in contrast, gave priority to ^{15}N accumulation in belowground and stem tissues and predominantly allocated ^{15}N to the green parts 1 year after label injection. If herbaceous plants are becoming more dominant as a consequence of climate changes, their high N uptake rates may lead to increased plant competitive strength toward microbes.

Nitrogen Uptake Responses in Microbes and Plants After Long-Term Warming and Fertilizer Addition

Warming did not affect the ^{15}N uptake by microbes, as also observed by Hobbie and Chapin (1998) in Alaskan tundra. However, in the experiment by Hobbie and Chapin, the greenhouses failed to raise the soil temperature, whereas the soil temperature in our experiment increased by 1.2–2.0°C (Michelsen and others 1996a; Rinnan and others 2007). Regardless of the raised soil temperatures, earlier studies at our experimental site have not revealed significantly increased N mineralization rates after warming (Schmidt and others 1999; Jonasson and others 2006), and, in agreement with this, we did not observe significantly increased levels of inorganic soil N, microbial N, or ^{15}N biomass. Neither did the microbial ^{15}N loss rate change with warming, indicating unchanged microbial turnover and mineralization rates.

The long-term fertilizer addition did not affect the microbial uptake of ^{15}N during the first few days after label addition (Figure 5B). Hence, the microbial demand for N appears not to be saturated by the substantial fertilization of the plots, and the microbes thus retained a large part of the added N in all treatments. However, after 1 year, the concentration of label in microbes tended to be lower in fertilized compared to unfertilized plots. This was

probably because the soil and microbial N pools were slightly higher after fertilization (Figure 2D; Rinnan and others 2007), of which follows that the dilution of ^{15}N in the soil increased and thereby reduced the microbial ^{15}N concentration. Hence, even though the initial uptake by the microbes in the fertilized plots did not decrease, the larger ^{15}N pool dilution 1 year after ^{15}N addition shows that more N is cycled in the fertilized than in the unfertilized plots. Thereby, the ^{15}N label addition method can be a valuable tool to demonstrate small changes in ecosystem N pools and turnover rates, which are difficult to detect with the traditional methods, for example, measurements of nutrient concentrations and mineralization rates by the buried bag technique.

We expected that most plants in fertilized plots would show lower uptake of the added ^{15}N, because they have been supplied with N during more than a decade with, in most years, annual additions greatly exceeding the estimated annual N uptake. It seems likely, therefore, that the additions should have relieved the plants from N limitation. Most functional plant groups also had increased their N pools in plots with long-term addition of fertilizer, but only the herbs and mosses changed the ^{15}N uptake in response to warming and fertilization. The ^{15}N concentration in herbaceous plants changed as we had expected, with reduced ^{15}N concentration in fertilized plots (Bassirirad 2000). The ^{15}N recovery did, however, not decrease correspondingly because the biomass increased, showing that the herbaceous species benefit from N fertilization by higher growth rates as a result of their potential for high uptake rates and allocation to aboveground plant parts.

Mosses had low uptake of ^{15}N in the fertilized plots, probably because of the saturated N demand as their N concentration in the long-term fertilized plots had doubled compared to that in unfertilized plots. However, even though moss growth no longer appears to be N-limited, the growth instead is restricted by increasing competition for light with vascular plants after increases of their biomass and canopy cover. Hence, the mosses thereby do not benefit from their opportunistic N uptake by increased production. The observed low biomass and ^{15}N recovery by mosses in warmed plots probably is a result of a significant drying of the soil surface (Robinson and others 1997), as the mosses lack roots and, consequently, cannot exploit deeper soil layers for nutrients and water.

Deciduous plants tended to show higher and evergreen plants lower ^{15}N recovery in warmed plots. This was largely a function of their changed biomass, indicated by unaffected tissue N concentration. This suggests that N was not diluted by temperature enhanced growth in the deciduous species and, hence, that the net N mineralization rate was sufficient to meet their increased demand for N. In contrast, the evergreens, with reduced biomass, also reduced the N uptake and kept unchanged tissue N concentrations.

Conclusions

This is the first study to concentrate on responses in uptake of added ^{15}N label to both long-term warming and fertilization in an arctic ecosystem. It is also one of a few studies that follow the ecosystem distribution of added label for a period longer than a few weeks. The treatments had little effect on microbial label uptake, and the microbes acted as strong sinks for the added ^{15}N in all treatments. Total plant ^{15}N recovery was not affected consistently among the different harvest occasions by the fertilization, but ^{15}N uptake by herbs and mosses was N saturated. Also, the level of inorganic soil N did not vary among treatments. Hence, the plants and microbes in both warmed and fertilized plots still had a high demand for N. The fate of the label in the ecosystem through 1 year shows that microbes initially immobilized most of the added label, but lost 2/3 of the acquired N during the following year, which indicates a relatively short turnover period of microbial N. Most of the ^{15}N was subsequently fixed in the SOM pool and this large accumulation of N in the SOM pool could partly explain the long response time of these ecosystems to induced climate change. We observed a continuous uptake of added ^{15}N in plants throughout the experiment period, implying that the plants are able to efficiently capture N, which continuously is released from the microbial biomass.

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