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Nitrogen Fixation, Denitrification, and Ecosystem Nitrogen Pools in Relation to Vegetation Development in the Subarctic

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Abstract

Nitrogen (N) fixation, denitrification, and ecosystem pools of nitrogen were measured in three subarctic ecosystem types differing in soil frost-heaving activity and vegetation cover. N₂-fixation was measured by the acetylene reduction assay and converted to absolute N ecosystem input by estimates of conversion factors between acetylene reduction and ¹⁵N incorporation. One aim was to relate nitrogen fluxes and nitrogen pools to the mosaic of ecosystem types of different stability common in areas of soil frost movements. A second aim was to identify abiotic controls on N₂-fixation by simultaneous measurements of temperature, light, and soil moisture.

Nitrogen fixation rate was high with seasonal input estimated at 1.1 g N m⁻² on frost-heaved sorted circles, which was higher than the total plant N content and exceeded estimated annual plant N uptake several-fold but was lower than the microbial N content. Seasonal fixation decreased to 0.88 g N m⁻² on frost-heaved moss-covered surfaces and to 0.25 g N m⁻² in stable heath vegetation, both lower than the plant and microbial N content. Yet fixation was estimated to correspond to about 2.7 times the annual plant N demand on the moss-covered surfaces but less than the plants' demand on the heath. Surprisingly, we found no denitrification on any surface.

Climatic changes in the Arctic will generate a warmer climate and change precipitation patterns. A warmer, drier environment will decrease N₂-fixation and thereby N availability to plants and microorganisms, while wetter conditions probably will increase N₂-fixation and thereby N supply to the surroundings.

Introduction

Soils in many places in the Arctic are subjected to strong frost movements, creating characteristic, more or less symmetrical structures of patterned ground such as circles, polygons, and stripes (Washburn, 1979). Areas with strong movements, so called cryoturbation, are particularly prevalent on wet, fine-grained soils. They are sparsely vegetated due to disruption of the root systems of higher plants and have a poorly developed organic soil horizon. However, cryptogams are generally abundant and often form a blackish crust on the surfaces, in which nitrogen (N) fixing cyanobacteria are important components (Dickson, 2000). The cyanobacteria play a vital role in supplying nitrogen from the atmosphere to the ecosystems and are the principal sources of nitrogen input to the soils (Gold and Bliss, 1995; Gold, 1998). The rate of N₂-fixation is favored by the high soil moisture, temperature, and light levels (Chapin et al., 1991; Lennihan et al., 1994; Nash and Olafsen, 1995; Liengen and Olsen, 1997b; Zielke et al., 2002; Uliassi and Ruess, 2002) of the black crust and the usually circumneutral pH (Jonasson and Sköld, 1983; Jonasson, 1986). Indeed, nitrogen fixation on these surfaces often is high enough to meet the entire nitrogen needs of the low biomass and may even be high enough to sustain additional plant growth (Dickson, 2000). However, the potential for loss of N also is high, because the same conditions promoting N₂-fixation also are favorable for denitrification (Chapin, 1996), which is likely to reduce the ecosystem gain of N even under high rates of N₂-fixation.

As the surfaces stabilize, and on soils of lower frost heaving activity outside of the structures of pattern ground, vascular plants increase in abundance (Jonasson 1986), N₂-fixation usually declines (Crocker and Major, 1955; Blundon and Dale, 1990), and the

proportion of plant growth that can be met by N₂-fixation is reduced. The activity of the cryoturbation is primarily dependent on the hydrology (Anderson and Bliss, 1998). After formation of the cryptogamic crust, drying of the soils stabilizes the surfaces and enhances colonization by mosses, lichens, and vascular plants (Gold, 1998; Bliss and Gold, 1999) with a further stabilization of the surfaces (Gold and Bliss, 1995; Hodkinson et al., 2003). Current changes in the arctic climate with increasing temperatures (IPCC, 2001) are likely to stabilize the soils of many areas with presently high cryoturbation activity. As a consequence, the N₂-fixation is also likely to decrease and the balance between gains and losses of N may also change depending on the effects on denitrification (Christensen et al., 1999).

The objective of this study was, first, to estimate nitrogen exchange as the balance between nitrogen fixation and denitrification on three surfaces in an area with a mosaic of actively frost-heaved and poorly vegetated sorted circles surrounded by surfaces of lower disturbance and non-disrupted vegetation. A second objective was to relate the flux rates to abiotic factors in order to determine principal controls on N₂-fixation among different vegetation types. Third, we measured the principal ecosystem pools of N in plants and soils to estimate the proportion of N entering into and disappearing from the vegetation types relative to the amounts already present.

Methods

SITE DESCRIPTION

Field work took place at the shore of Lake Torneträsk, Abisko, north Sweden (68°19'N, 18°51'E), with a long term (30 yr) annual mean temperature of -0.5°C, average July temperature of 11°C, and mean annual precipitation of 304 mm.

Within an area of about 300×200 m, we selected eight sampling plots in each of three vegetation types representing different stages of soil disturbance and vegetation cover (Jonasson, 1986). The most disturbed plots were at the center of actively frost-heaved sorted circles (Washurn, 1979) of about 4–7 m diameter covered by a thin organic crust layer but with little or no coverage of mosses and lichens. Higher plants occurred scattered, and the dominant species were the graminoids *Juncus triglumis* and *Carex microglochin*, the herbs *Pinguicula vulgaris* and *Tofieldia pusilla*, and the vascular cryptogam *Equisetum scirpoides*. Contrasting to this, we placed a second series of plots on drier, stable ground outside the circles in heath vegetation with a well-developed organic horizon. The shrub *Betula nana* and the ericaceous dwarf shrubs *Empetrum hermaphroditum* and *Vaccinium uliginosum* dominated and overtopped scattered mosses, liverworts, and lichens, among others the N_2 -fixing *Peltigera aptosa*. The third series of plots was placed just outside the circles on less disturbed but yet frost-heaved ground (Jonasson 1986) with a well-developed organic horizon but with only scattered vascular plants of mostly *V. uliginosum*, *Arctostaphylos alpina*, and *E. hermaphroditum*. The ground was covered by thick moss mats with scattered lichens, mostly *P. aptosa* and *P. venosa*. These plot types are hereafter called the circle, the heath, and the moss plots.

Heath vegetation dominates the area above the treeline and in treeless openings in the birch forest at lower altitudes. Sorted circles and unstable moss-rich patches are scattered in the heath vegetation and are common particularly at level ground.

SAMPLING OF N_2O AND ASSAY OF N_2 -FIXATION

Denitrification was measured as nitrous oxide (N_2O) fluxes from the soil to the atmosphere, and nitrogen fixation was estimated by the acetylene reduction method (Stewart et al., 1967). The measurements were done by the closed chamber technique (Christensen et al., 1995). Aluminum chamber bases of 506 cm^2 basal area were pushed into slots cut into the soil to 10 cm on the heath and moss plots and to 3–5 cm in the stone-rich circle plots to avoid escape of gases. Acrylic chambers of 8 L were fitted to a water channel on the top of the bases to ensure an airtight seal. The chambers had a rubber septum on the top through which a syringe could be inserted, and gases could be extracted from the chamber atmosphere. In order to avoid warming the chambers by direct sunlight, they were covered with sackcloth on clear days. We also measured soil temperatures to 2 cm soil depth inside the chambers, i.e., in the horizon where most N_2 -fixation took place, without noting any influence of the chambers on the temperature during the 2 h of measurement (data not shown).

For estimation of N_2O production, we extracted syringe samples of 50 mL chamber air immediately after positioning the chambers on the bases and thereafter at two 60 min intervals. The samples were immediately transferred into evacuated serum bottles of 30 mL volume and kept until analysis. Nitrous oxide samples were taken on 30 July, 2 and 28 August, 1 and 5 September 2001.

Acetylene reduction is an indirect method used to estimate nitrogen fixation (Stewart et al., 1967). Acetylene (C_2H_2) is reduced to ethylene (C_2H_4) by nitrogenase in the N_2 -fixing microbes. Nitrogenase normally reduces N_2 to ammonia, but will also reduce other compounds with a triple bond. Acetylene reduction was measured as soon as possible after the N_2O sampling, i.e., on 30 July, 3 and 28 August, 2 and 6 September, in the same chambers, and additional measurements were done on 21, 25, and 28 June. We placed a container with calcium carbide (CaC_2) inside the chambers and injected water to the carbide through the rubber septum to release acetylene. The amount of carbide was adjusted to release approximately 10% v/v acetylene to the chamber atmosphere (chamber volume ~ 8 l and 2.1 g CaC_2). Syringe samples of 5 mL were taken after 3 min following the injection of water, and thereafter at two 60 min intervals. The syringes were sealed

with a rubber stopper until analysis of ethylene and acetylene, which took place within 30 h after the sampling. Acetylene was measured 3 min following injection of water, in order to ensure that the chamber concentration was at least 10% v/v. Furthermore, acetylene concentrations also were measured after 60 and 120 min and were used as internal standards to check for chamber leakages. After each assay of acetylene reduction, the chambers were moved to a new position, because acetylene inhibits nitrification (Bollman and Conrad, 1997) and could reduce the rate of substrate formation for denitrification.

On the heath plots, we found that acetylene escaped from the chambers because dry conditions created a poor seal between the chamber bases and the soil. We therefore dug up the entire soil column within the chambers, including plants, sealed the bottom of the chamber base with a polyethylene sheet, after which we replaced the chambers with the soil and did the measurements. On the circle and moss plots, the soil moisture provided a much better seal and the losses of acetylene were low. To examine temperature effects on the processes, we measured temperature in 2 cm depth with four Tinytag Plus data loggers (Gemini Data Loggers UK Ltd., U.K.) per vegetation type every 10 min between 25 June and 6 September. Data for levels of incoming light and air temperature (10 min intervals) were provided by a meteorological station at Abisko Scientific Research Station ~ 500 m from the study site.

Nitrous oxide concentrations were measured on a Shimadzu GC-17A gas chromatograph with a Supelco Carboxen column and with injector, column, and detector oven temperature at 80, 120 and 315°C , respectively, using N_2 as carrier gas. Acetylene and ethylene concentrations were measured on a Shimadzu GC-14B gas chromatograph with a Porapak N column and with injector, column, and detector oven temperature at 250, 65, and 120°C , respectively, using He as carrier gas.

SOIL AND PLANT SAMPLING AND ANALYSIS

Immediately after each assay of acetylene reduction, cores of 4 cm diameter were collected from the organic horizon inside the chamber bases to a depth of 10 cm on the moss and heath plots and to 2 cm on the circles, where the organic horizon was much shallower. Additional soil samples were taken on 31 August from all surfaces as above + from 2–5 cm and from the organic crust on the circles by scraping off the upper layer with a knife. The soil was kept at 4°C , and within 24 h it was passed through a 2 mm sieve. Soil water content was measured gravimetrically, and a part of the dried soil was ashed at 550°C for determination of loss on ignition. The remaining soil was used for determination of microbial N, dissolved organic N, NH_4^+ , and NO_3^- .

Total C and N were measured on a EuroVector CN analyzer on the soil sampled 31 August. Five grams of fresh soil were 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 25 mL of $0.5 \text{ mol L}^{-1} K_2SO_4$ (Brooks et al., 1985). The extracts were filtered through Whatman GF-D filters and frozen until analysis. Another 5 g of fresh soil were treated as above, but without fumigation, to recover soil inorganic N. To obtain dissolved organic N, 10 mL of K_2SO_4 extract were digested with 5 mL concentrated H_2SO_4 with H_2SeO_3 and 1 mL 30% H_2O_2 added. NO_3^- -N was analyzed by the cadmium reduction method with a flow injection analyzer, and NH_4^+ -N was analyzed spectrophotometrically by the indophenol method. Dissolved organic N was calculated by subtracting the N in digested, unfumigated extracts from that in undigested, unfumigated extracts. The microbial N content was analyzed 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 (Jonasson et al., 1996).

After measurement of N_2O -release and the acetylene reduction assay on 30 July, we harvested all above-ground vegetation within the

chambers. After sorting the vegetation into species it was dried at 80°C, weighed, and analyzed for total N using a LECO FP-428 N analyzer.

CONVERSION OF ETHYLENE PRODUCTION TO NITROGEN FIXATION

The theoretical conversion factor between ethylene produced to nitrogen fixed is between three and four (Hardy et al., 1973; Jensen and Cox, 1983). However, in experiments comparing ethylene production to incorporation of $^{15}\text{N}_2$, the observed conversion factor differs from the theoretical factor (Liengen, 1999a) due to, e.g., higher solubility of acetylene than nitrogen in water (Rice and Paul, 1971) or adsorption of acetylene to soil colloids (Rennie et al., 1978; DeLuca et al., 2002). Therefore, it is necessary to estimate the conversion factor separately for each type of surface at which the fixation is measured.

We did this by collecting five soil samples (each 19.6 cm²) from the upper 1 cm on each plot, which we incubated with acetylene in the laboratory for assay of ethylene production, followed by incubation with $^{15}\text{N}_2$ and measurement of the direct incorporation of N_2 through N_2 -fixation. We used soils from the upper 1 cm because previous assays in which we compared the ethylene production in the soil horizons between 0 and 1 cm, 1 and 5 cm, and 5 and 10 cm depth had shown ethylene production activity in the 0–1 cm horizon only (data not shown). The samples were placed in 500 mL containers and closed with a rubber septum after which we replaced 10% of the headspace volume with acetylene. The samples were incubated for 2 h at 18°C and the light varied between 500 and 700 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Because the diurnal mean temperature across the dates of measurements was 13.5°C, we estimated that 18°C was a realistic average chamber temperature during midday and afternoon when we did the field samplings. Samples were taken after 3, 63, and 123 min and analyzed for ethylene as for the field measurements. After the acetylene reduction assay, the containers were left open for 3 h to allow all acetylene to escape. We thereafter closed the lids and replaced 5% (v/v) of the headspace gas volume with $^{15}\text{N}_2$ (98% $^{15}\text{N}_2$, Cambridge Isotope Laboratories, Inc., U.S.A.) and incubated the samples for 2 h as above. After the incubation, the samples were dried at 80° before analysis of their ^{15}N content (EuroVector CN analyzer coupled to a Micromass IsoPrime Isotope Ratio Mass Spectrometer). The incorporation rate was estimated by comparison of the ^{15}N concentration in samples incubated with and without $^{15}\text{N}_2$, and the amount of nitrogen fixed was calculated using the equation given by Liengen (1999a).

ESTIMATIONS OF SEASONAL N_2 -FIXATION

The total amount of nitrogen fixed at the different surfaces during the period of measurement from 21 June to 6 September was estimated from the mean hourly values of ethylene production at two succeeding measurements. We multiplied the production with the actual day lengths (data provided by the Abisko Scientific Research Station) and with the number of days between the measurements. The production of ethylene was converted to fixed nitrogen using the surface-specific conversion factors.

STATISTICAL ANALYSIS

All data were tested by Levene's test of homogeneity of variances and, if necessary, transformed. One-way ANOVAs with type of surface or date as factors followed by Tukey's test were used to test for differences in ethylene production, soil ammonium, dissolved organic N, and soil microbial N among type of surfaces or time of measurements. Spearman correlations were used to test for relationships between nitrogen fixation and soil temperatures, soil moisture, and

TABLE 1

Dry weight biomass (g m^{-2}) \pm SE for plant life forms on the heath, the moss, and the circle surfaces ($n = 8$) on 1 August 2001.

	Heath	Moss	Circle
Mosses	75.1 \pm 13.5	200.3 \pm 13.8	0 \pm 0
N-fixing <i>Peltigera</i> sp.	8.6 \pm 3.5	13.9 \pm 8.5	0 \pm 0
Graminoids	6.3 \pm 2.2	7.5 \pm 2.3	14.7 \pm 7.5
Herbs	7.5 \pm 2.5	13.9 \pm 4.0	4.9 \pm 1.4
Deciduous dwarf shrubs	60.7 \pm 9.2	55.4 \pm 9.7	3.3 \pm 2.1
Evergreen dwarf shrubs	212.7 \pm 28.6	26.9 \pm 8.5	0.1 \pm 0.1
Total biomass	370.9 \pm 29.5	317.9 \pm 32.1	23.3 \pm 8.2

light levels. All statistical tests were carried out with SAS (Statistical Analysis System Institute, 1997).

Results

VEGETATION CHARACTERISTICS OF THE CIRCLES, HEATH, AND MOSS PLOTS

The surface of the circles was almost completely covered by a blackish crust or sheet-forming colonies of N_2 -fixing *Nostoc* species. The biomass of vascular plants was only about 23 g m^{-2} (Table 1) with graminoids making up about 60%. The biomasses on the moss and heath surfaces were about 320 g m^{-2} and 370 g m^{-2} , with mosses making up 63% of the total biomass on the moss plots and dwarf shrubs making up 84% of the total biomass on the heath plots. The N_2 -fixing lichens, *Peltigera aptosa* and *P. nervosa*, occurred in relatively large amounts on both the moss and the heath plots, and the legume, *Astragalus alpinus*, with N_2 -fixing rhizobia occurred scattered with a low biomass of 0.4 g m^{-2} on the moss plots and 0.1 g m^{-2} on the circles. The biomass of cyanobacteria of mostly microscopic species that occurred mixed with the soil and on the vegetation was not possible to estimate.

ENVIRONMENTAL PARAMETERS AND SOIL CHARACTERISTICS

Diurnal mean air temperature across the days of measurement was 13.5°C. The temperature was high in the beginning of the measurement period, with a maximum of 19.2°C on 9 July (Fig. 1). From mid-July and during the rest of the period, the air temperature varied around 10–12°C. However, the temperature at the exact time of acetylene reduction often exceeded the diurnal means because measurements were done during daytime. Incoming light varied between 121 and 1140 $\mu\text{mol photons m}^{-2} \text{ h}^{-1}$ during the measurements with a mean of 550 $\mu\text{mol photons m}^{-2} \text{ h}^{-1}$ (Fig. 1).

On the heath and the moss plots, the depth of organic horizon was 9.6 and 9.3 cm, respectively, with a distinct limit to the underlying inorganic soil. The loss on ignition was 88% on the heath and 82% on the moss plots, indicating little mixing of mineral soil with the organic matter. On the circles, the organic horizon only reached 2 cm depth and was mixed with mineral soil, shown in Table 2 by a significantly lower loss on ignition (of about 20%) than of the soils from the two other surface types. The samples of the organic crust from the circles contained 31.4 \pm 2.7% C and 1.2 \pm 0.1% N, which was significantly higher than the C and N content of 4.97 \pm 0.7% and 0.42 \pm 0.06%, respectively, of the underlying 2-cm-deep soil horizon and the C content of 3.6 \pm 1.1% and the N content of 0.1 \pm 0.06% in the horizon between 2 and 5 cm depth.

Soil pH increased from 5.9 at the heath to 7.5 on the circles, with an intermediate value of 7.0 on the moss surfaces (Table 2). The soil water content ranged from 130% of the dry mass on the circles with

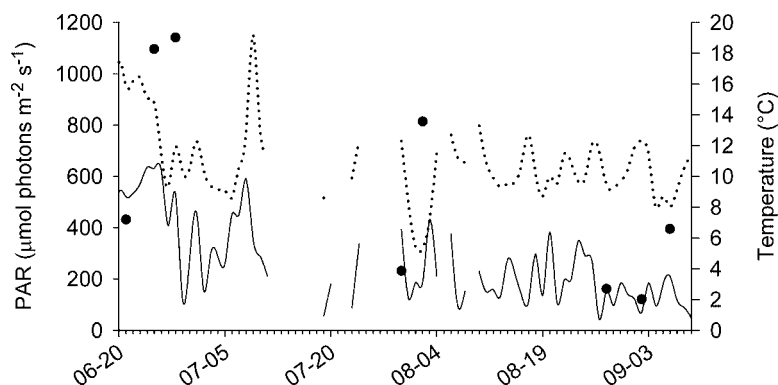


FIGURE 1. Daily mean air temperature (dotted line) and incoming photosynthetically active radiation (PAR) (solid line) at Abisko during the 2001 season. Incoming light at the time of acetylene reduction assay are shown with black dots (●). The gaps were caused by technical problems at the meteorological station at Abisko Scientific Research Station.

high content of mineral soil to more than 500% at the moss plots with much higher organic matter content, while the heath had intermediate values, despite the higher content of organic matter (Fig. 2, Table 2). There were only significant (one-way ANOVA, $F = 3.51$, $P = 0.0035$) changes in soil water content over the growing season on the moss surfaces with the water content ranging from 402 to 629% of the dry mass (Fig. 2).

POOLS AND DISTRIBUTION OF NITROGEN

The total C and N content in the organic horizons, expressed as content per m^2 , differed significantly among type of surfaces, being highest on the heath and lowest on the circles (Table 2). Of the inorganic N fractions, NO_3^- -N was below the detection limit of about $0.05 \mu g g^{-1}$ on all surfaces and at all sampling dates. Extractable NH_4^+ -N was significantly higher on the moss plots than on the other plots, but did not differ significantly between the heath and circles despite the much lower organic matter content on the circles (Fig. 3a). The content of dissolved organic nitrogen and microbial nitrogen were, however, significantly lower on the circles than on the two other surfaces (Figs. 3b and 3c). Regardless of the much lower organic matter content on the circles, microbial N content did not differ much, due to high concentrations per unit dry soil mass.

The pools of plant and microbial biomass N plus labile N made up 3.9, 6.2, and 7.5% of the total N at the heath, the moss, and the circles, respectively. The plant N made up roughly 44% and the microbial N 47% of the total pool of biomass + labile N at both the heath and the moss plots (Fig. 4). On the circles, the distribution was different, however, as microbial N made up 85% and plant N only 7% of the total pool (Fig. 4).

N_2O FLUXES AND ETHYLENE PRODUCTION

We did not find nitrous oxide emissions on any measurement occasion, but with the acetylene reduction assay we found ethylene

production on all type of surfaces and on all dates of measurements, indicating N_2 -fixation activity. The production varied, however, strongly and significantly ($P \leq 0.0001$, $F = 119.5$) from a seasonal average of $3.5 \mu mol ethylene m^{-2} h^{-1}$ on the heath plots, to a tenfold increase of $32.6 \mu mol ethylene m^{-2} h^{-1}$ on the circles, while the production on the moss plots was intermediate at $17.3 \mu mol ethylene m^{-2} h^{-1}$ (Fig. 5). The ethylene production on the moss and circle plots differed significantly during the summer ($F = 2.50$, $P = 0.028$; and $F = 5.58$, $P \leq 0.0001$, respectively) with highest rates in June, leveling off until September. In contrast, there was no significant seasonal variation of ethylene production on the heath plots ($F = 1.10$, $P = 0.379$).

The ethylene production on the circles and moss plots correlated significantly with incoming photosynthetically active radiation (PAR) ($r_s = 0.81$, $P = 0.015$; $r_s = 0.95$, $P < 0.001$, respectively) and soil temperature at 2 cm depth ($r_s = 0.85$, $P = 0.016$; $r_s = 0.86$, $P = 0.012$, respectively) (Fig. 6). However, PAR and soil temperatures were also significantly intercorrelated, with correlation coefficients of between 0.71 and 0.85 on the three surfaces. On the circles, but not on the two other surface types, the ethylene production also correlated significantly with soil water content ($r_s = 0.71$, $P = 0.047$) (Fig. 6).

SEASONAL NITROGEN FIXATION

The conversion factor for ethylene produced to nitrogen fixed was determined to be 1.63 on the circles, 2.48 on the moss plots, and 4.22 on the heath plots. Using the surface-specific conversion factors, we estimated the seasonal (21 June–6 September) nitrogen fixation per m^2 to $0.25 g N m^{-2}$ on the heath plots, $0.88 g N m^{-2}$ on the moss plots, and to $1.1 g N m^{-2}$ on the circles.

Discussion

NITROGEN FIXATION AND DENITRIFICATION IN RELATION TO ECOSYSTEM DEVELOPMENT

We did not detect denitrification on any of the surfaces, which agrees with lack of denitrification in the only other study we are aware of of denitrification activity on frost-heaved ground (Gersper et al., 1980). However, at meadow sites in the Arctic, denitrification rates have been measured at between 16 and $99 \mu g N m^{-2} d^{-1}$ (Gersper et al., 1980; Chapin, 1996). The lack of denitrification on our plots could be because the soil did not contain any detectable nitrate due to low production rate, or because formed nitrate rapidly was leached from the plots with drainage water, causing substrate limitation. The lack of denitrification is in accordance with a study at a heath site a few kilometers from the sites of this study, where denitrification was detected only when soils were fertilized with NH_4NO_3 but not with organic N as glycine (Christensen et al., 1999). We only measured denitrification during summer, while studies in alpine meadows show

TABLE 2

Some soil physical and chemical characteristics on the heath, moss, and circle surfaces. Means \pm SE. Data for pH, LOI, and soil water are across all sampling occasions ($n = 64$), data for total C and total N are from 31 August ($n = 8$). Significantly different means among vegetation types after Tukey's test ($P = 0.05$) are shown with different letters.

	Heath (upper 10 cm)	Moss (upper 10 cm)	Circle (upper 2 cm)
pH (H_2O)	5.9 ± 0.2 C	7.0 ± 0.1 B	7.5 ± 0.1 A
LOI (% of dw)	87.9 ± 0.9 A	82.3 ± 1.7 B	21.2 ± 1.4 C
Soil water (% of dw)	330 ± 12 A	514 ± 20 A	131 ± 8 B
Soil mass ($g m^{-2}$)	10567 ± 438 A	8705 ± 601 A	10654 ± 1244 A
Total C ($g m^{-2}$)	5804 ± 759 A	3726 ± 372 B	529 ± 72 C
Total N ($g m^{-2}$)	192 ± 20 A	121 ± 17 B	45 ± 6 C

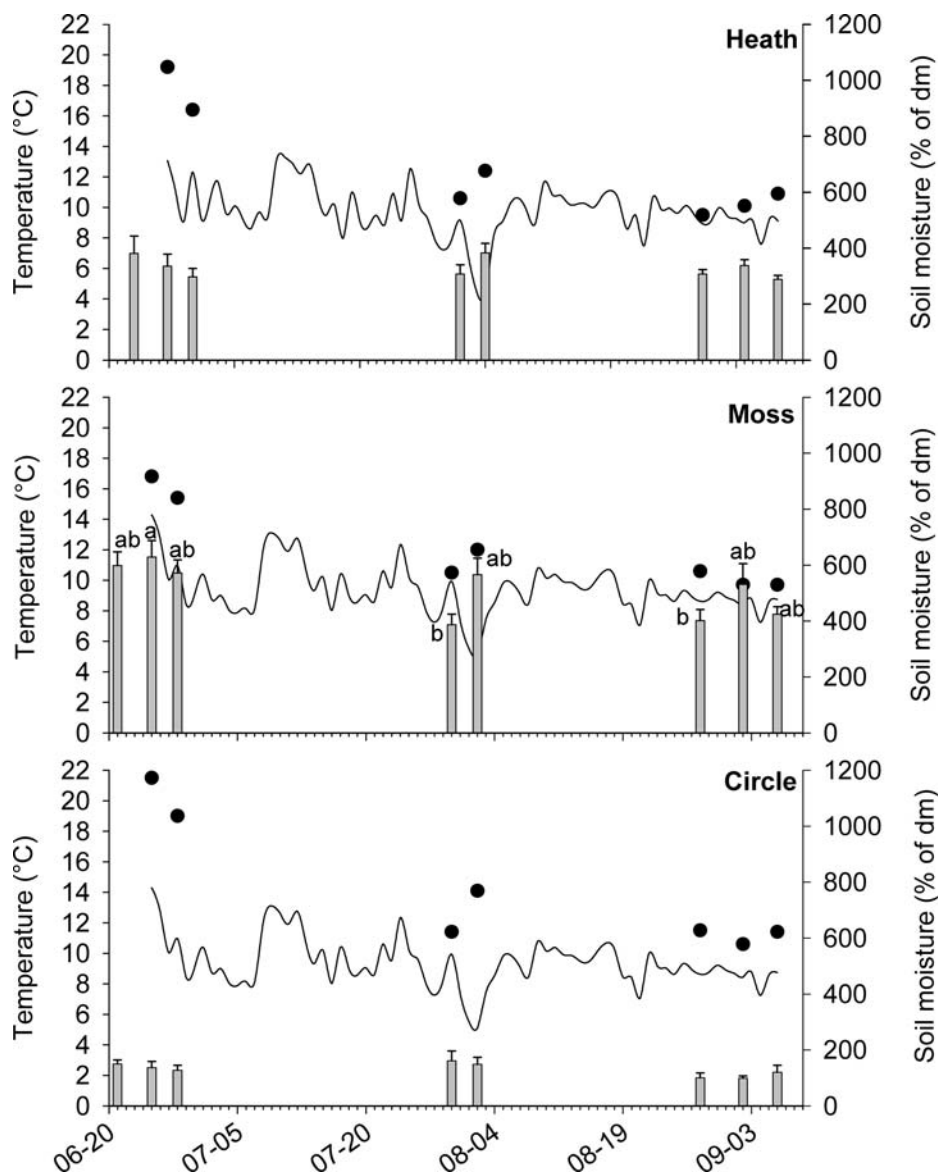


FIGURE 2. Diurnal means of soil temperature in 2 cm depth on the 8 days of acetylene reduction assay on the heath, moss, and circle plots and soil moisture content. Black dots are the soil temperature during acetylene reduction assays, and the black lines are the diurnal means for each day (loggers installed 25 June). Soil moisture data (bars) are means \pm SE, $n = 8$. Significantly different means of soil moisture at the days of measurement are shown with different letters (Tukey's test, $P < 0.05$).

that denitrification can be substantial during winter (Williams et al., 1998), and in incubation experiments with agricultural soils also when soils are exposed to repeated freeze-thaw cycles (Chen et al., 1995; Teepe et al., 2001). Hence, we cannot rule out that denitrification might take place at our sites outside of the summer season.

The measured ethylene production rates in this study are comparable to reported rates in other studies in Arctic and Antarctica on crusted ground (Alexander et al., 1978; Liengen and Olsen, 1997a; Dickson, 2000) and in areas with moss-*Nostoc* associations (Alexander et al., 1978; Davey and Marchant, 1983; Karagatzides et al., 1985; Chapin et al., 1991; Solheim et al., 2002).

We found highest rates of ethylene production on the circles, with the lowest plant biomass and a high cover of cryptogamic crust. On the moss plots with more vegetation and a high cover of mosses with possible associations with cyanobacteria, ethylene production was significantly lower. On the heath plots, with the highest vascular biomass but low moss biomass, the ethylene production was low and probably associated with presence of *Peltigera* sp. Hence, the nitrogen fixation decreased with increasing vegetation development, which is in agreement with other studies in the Arctic showing decreasing nitrogen fixation with advancing succession and increasing plant cover (Crocker and Major, 1955; Liengen and Olsen, 1997b).

ABIOTIC CONTROLS ON N_2 -FIXATION

We found significant linear correlations between ethylene production and soil temperature at 2 cm depth on both the moss and circle plots (Fig. 6), which is in accordance with reported positive relationship between ethylene production and temperature up to 20–32°C from incubation studies under controlled conditions (Alexander and Schell, 1973; Lennihan et al., 1994; Liengen, 1999b; Dickson, 2000; Zielke et al., 2002). The diurnal temperature always was below 20°C during the summer and we only recorded one case of temperature above this level at the measurement occasions (Fig. 2), indicating that the nitrogen fixation at our sites probably is limited by temperature during most of the summer season.

The ethylene production rates on the circles and moss surfaces also correlated positively with PAR. However, other studies in the Arctic have shown light saturated ethylene production at PAR levels between 100 and 150 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Lennihan et al., 1994; Zielke et al., 2002). It is likely, therefore, that the ethylene production rates in our study were light saturated at all measurement occasions, as the lowest amount of incoming light was 121 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 2). Because of the strong correlation between light and temperature, this implies that the significant correlations between ethylene

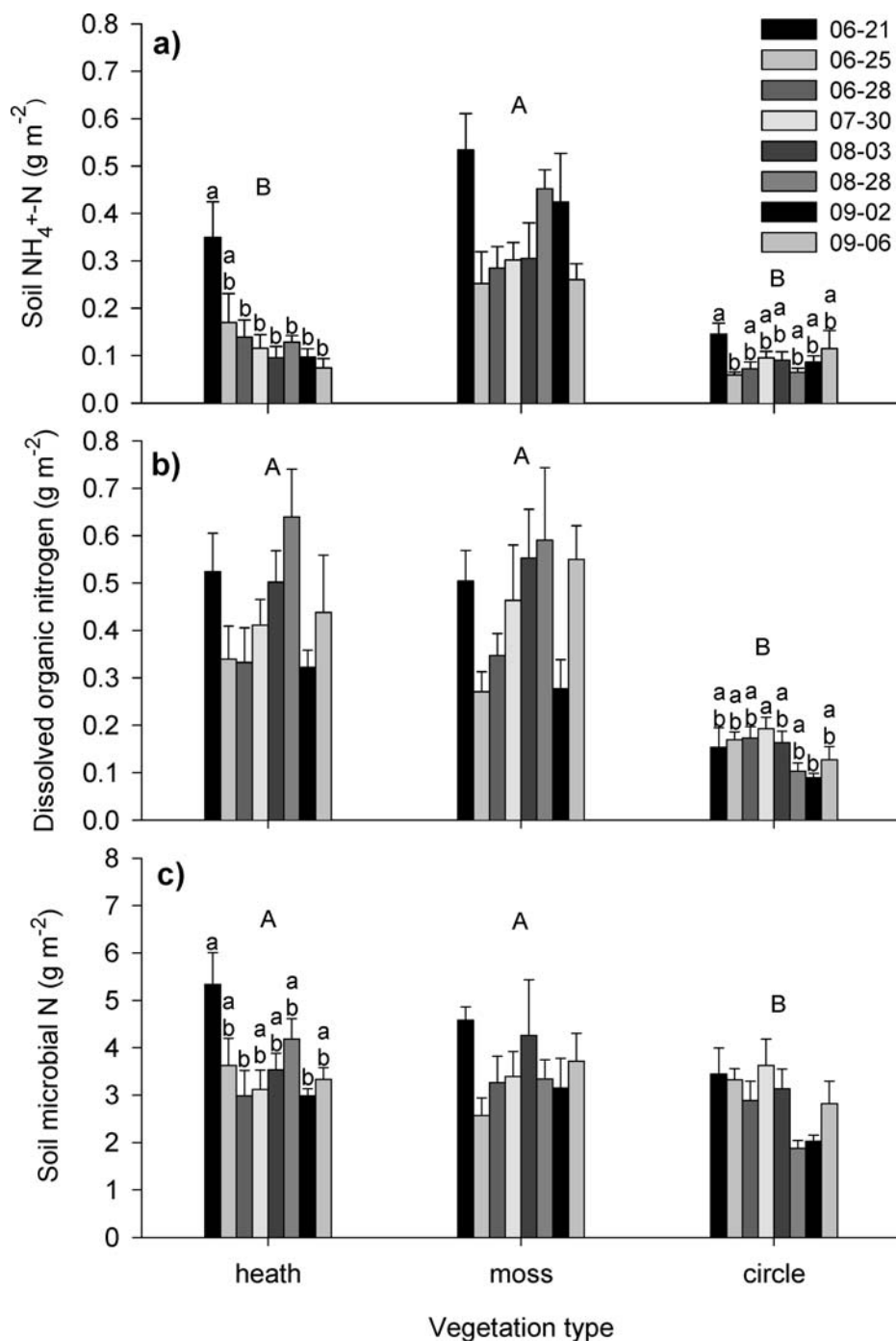


FIGURE 3. Variations in the pools of (a) Soil $\text{NH}_4^+\text{-N}$, (b) dissolved organic N, and (c) soil microbial N, at the 8 days of acetylene reduction assay on the heath, moss, and circles surfaces. Data are means \pm SE, $n = 8$. Significantly different means within vegetation type and sampling time are shown with different upper- and lowercase letters, respectively (Tukey's test, $P < 0.05$).

production and light that we observed were due to temperature rather than to light control.

Studies in arctic semi-deserts have shown that soil moisture has a strong control on ethylene production (Chapin et al., 1991; Liengen and Olsen, 1997b; Dickson, 2000; Zielke et al., 2002). We found correlations between ethylene production and soil moisture only on the moss surfaces. The lack of correlation on the two other surfaces probably was because these had a stable water content across all occasions of measurement (Fig. 2), which reduced the possibility to detect any correlation. However, we found a significant correlation between ethylene production and the soil water content per gram soil organic matter on the circles ($r_s = 0.71$, $P = 0.047$), presumably because of a more sensitive test when the effect of variable soil mineral matter was removed.

The lack of correlation between ethylene production and measured environmental variables on the heath surfaces probably is because of two

main reasons. First, the ethylene production was very close to the detection limit, which obscured the detection of any correlation. Second, and probably even more important, the N_2 -fixation at the heath probably was from fixing lichens with scattered occurrence across the plots, resulting in a high variation that did not depend on environmental factors. In contrast, on the circle and the moss plots, N_2 -fixation probably was from much more evenly distributed occurrences of soil- and moss-dwelling cyanobacteria, giving less "background noise" to the estimates.

SEASONAL NITROGEN FIXATION AND ECOSYSTEM SUPPLY

By using the surface-specific conversion factors, we estimated a seasonal (late June to early September) nitrogen fixation at 0.25 g N m^{-2} on the heath plots, 0.88 g N m^{-2} on the moss plots, and 1.1 g N m^{-2} on the circles. The amounts of nitrogen fixed on the surfaces in

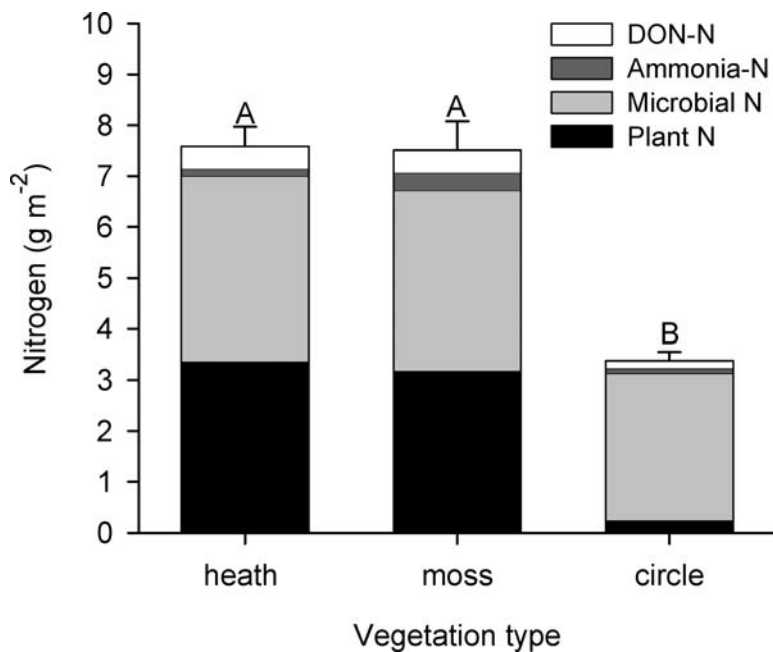


FIGURE 4. Average pools of plant N, microbial N, ammonia, and dissolved organic nitrogen (DON) for the period 21 June–6 September 2001, on the circle, the moss, and the heath surfaces. Standard errors are shown for the totals. Significantly different means of vegetation type are shown with different letters (Tukey's test, $P < 0.05$).

this experiment are probably underestimated since there was high fixation both at the first and last day of our measurements. This implies that the season for fixation is longer than the period between late June and early September, when we did the measurements, but should not severely influence the relative magnitude of the fixation between the three ecosystem types. Despite a probable underestimation and the rough estimation we did, the seasonal nitrogen fixation on our plots are comparable to estimated N fixation rates of $0.05\text{--}0.3\text{ g N m}^{-2}\text{ yr}^{-1}$ in different arctic (Alexander and Schell, 1973; Chapin et al., 1991; Dickson, 2000) and antarctic (Davey and Marchant, 1983) vegetation types, but considerably lower than the $12\text{ g N m}^{-2}\text{ yr}^{-1}$ estimated by Granhall and Lid-Torsvik (Granhall and Lid-Torsvik, 1975) in a wet minerotrophic mire at Stordalen, Abisko. In northern boreal forests, associations of *Nostoc* and the moss *Pleurozium schreberi* fixed $0.17\text{ g N m}^{-2}\text{ yr}^{-1}$ (DeLuca et al., 2002), calculated on basis of an estimated conversion factor of three for ethylene produced to nitrogen fixed.

N-fixing organisms lose fixed N to the surroundings (Millbank, 1982; Millbank and Olsen, 1986), mostly as nitrate and ammonium (Belnap, 2001) that can be sequestered by soil microorganisms and plants. The amount of fixed N on the circles is higher than the total aboveground pool of plant N (Fig. 4) and, hence, also higher than the annual plant uptake but lower than the microbial N pool. In contrast, on the moss and heath plots, N_2 -fixation rates are considerably lower than both the plant and microbial N content. Assuming that the plants take up $\sim 10\%$ of their N pool per year (Shaver and Chapin, 1991; Jonasson et al., 1999), the yearly nitrogen fixation corresponds with 74% and 2.7 times of the plants' nitrogen demand at the heath and the moss surfaces, respectively, while the amount of fixed nitrogen is 44 times higher than plant demand on the circles. It appears therefore that the N_2 -fixation rate by far exceeds the plant demand of the open vegetation on the circles and can supply the N to the vegetation on the moss-covered surfaces but supply N below the demand on the stable heath.

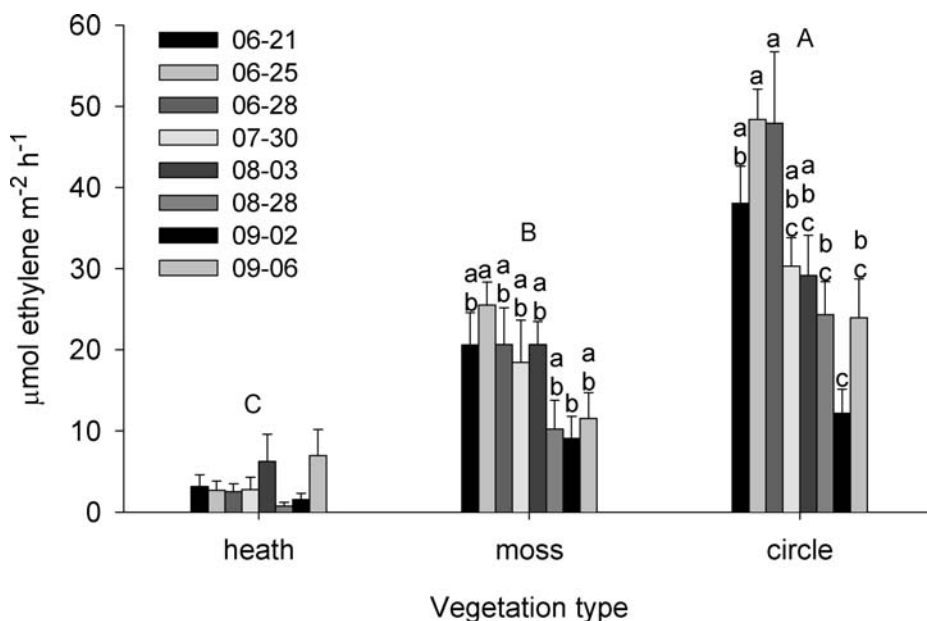


FIGURE 5. Ecosystem production of ethylene measured by acetylene reduction assays at different times during the growing season 2001 on the heath, the moss, and the circle plots. Data are means \pm SE; $n = 8$. Significantly different means of vegetation type and/or sampling time are shown with different upper- and lowercase letters, respectively (Tukey's test, $P < 0.05$).

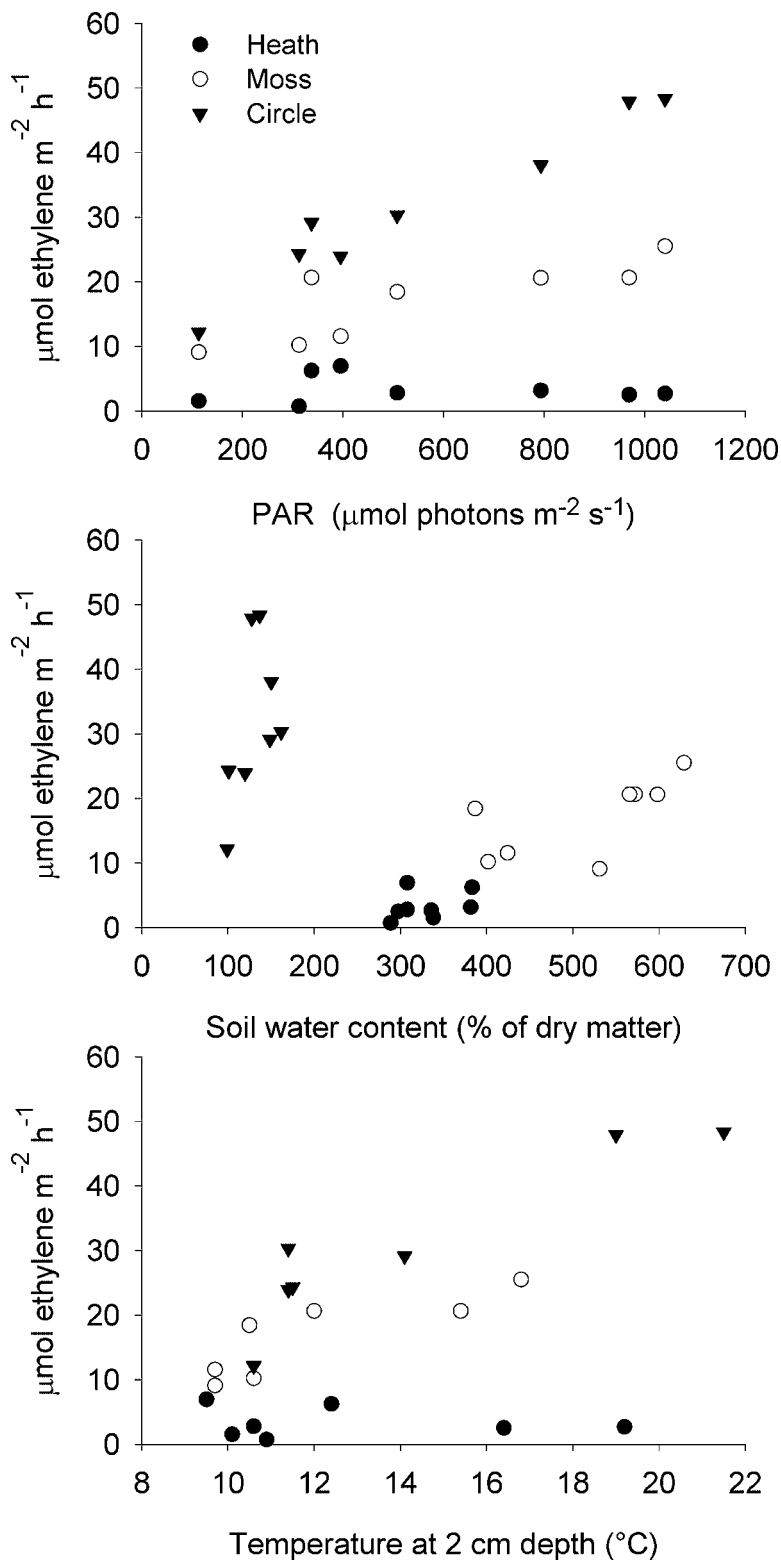


FIGURE 6. Scatter plots showing the relationships between ethylene production and PAR, soil water content, and temperature at 2 cm depth for the heath, moss, and circle surfaces.

Conclusions

Our measurements have shown, first, a strong control of nitrogen fixation rates by temperature and also a probable strong control by soil moisture, while light level during the summer presumably was above the saturation level during most of the time. Second, the spatial variation in fixing capacity was strongly affected by the vegetation type and degree of plant coverage, which in turn were related to the soil stability. Third, the seasonal nitrogen fixation on the least

vegetated surfaces was many-fold higher than the plants' demand. Surprisingly, we found no denitrification, possibly because of rapid drainage of nitrate, the substrate for denitrification, at least in the wet ecosystem types.

In a future warmer climate with changed precipitation (IPCC, 2001) and local changes in hydrological conditions (ACIA, 2004), many of the presently frost-heaved surfaces are likely to dry. This will stabilize the soil, increase the biomass of higher plants and contribute to further drying of the soil. On the other hand, the warming also will

cause melting of permafrost in some areas, which in combination with increased precipitation and locally impeded drainage (ACIA, 2004) will create wetter surfaces than at present.

Given the large differences in N₂-fixation rates we have shown between adjacent types of surfaces, the local effects on the N-cycle are likely to be particularly pronounced in areas undergoing great changes in soil moisture conditions and stability. N₂-fixation probably will decrease in areas subjected to drying, while it is likely to increase in areas becoming wetter and destabilized. If the denitrification rate is low, as in our study, and if most of the fixed N from the surfaces of high N₂-fixing activity is transported to the surroundings by drainage water, the changes are likely also to affect the entire watershed: In the surroundings of areas undergoing drying and stabilization, the N supply will decrease, while it will increase in areas subjected to wetting and destabilization.

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