

 Open access • Journal Article • DOI:10.1657/1523-0430(2006)38[363:NFISSA]2.0.CO;2

Nitrogen Fixation in Surface Soils and Vegetation in an Arctic Tundra Watershed: A Key Source of Atmospheric Nitrogen — Source link

Satoru Hobara, Carmody McCalley, Keisuke Koba, Anne E. Giblin ...+3 more authors

Institutions: National Institute for Environmental Studies, Marine Biological Laboratory, Tokyo Institute of Technology, Cornell University

Published on: 01 Aug 2006 - Arctic, Antarctic, and Alpine Research (Institute of Arctic and Alpine Research (INSTAAR), University of Colorado)

Topics: Tundra, Light intensity, Growing season, Watershed and Soil water

Related papers:

- [Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization](#)
- [Nitrogen Fixation in the High Arctic: Role of Vegetation and Environmental Conditions](#)
- [Responses of Arctic Tundra to Experimental and Observed Changes in Climate](#)
- [Effects of temperature and substrate quality on element mineralization in six arctic soils](#)
- [The Influence of Abiotic Factors on Biological Nitrogen Fixation in Different Types of Vegetation in the High Arctic, Svalbard](#)

Share this paper:    

View more about this paper here: <https://typeset.io/papers/nitrogen-fixation-in-surface-soils-and-vegetation-in-an-117zmjoc7j>

Nitrogen Fixation in Surface Soils and Vegetation in an Arctic Tundra Watershed: A Key Source of Atmospheric Nitrogen

Authors: Hobara, Satoru, McCalley, Carmody, Koba, Keisuke, Giblin, Anne E., Weiss, Marissa S., et al.

Source: Arctic, Antarctic, and Alpine Research, 38(3) : 363-372

Published By: Institute of Arctic and Alpine Research (INSTAAR),
University of Colorado

URL: [https://doi.org/10.1657/1523-0430\(2006\)38\[363:NFISSA\]2.0.CO;2](https://doi.org/10.1657/1523-0430(2006)38[363:NFISSA]2.0.CO;2)

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Nitrogen Fixation in Surface Soils and Vegetation in an Arctic Tundra Watershed: A Key Source of Atmospheric Nitrogen

Satoru Hobara*

Carmody McCalley†

Keisuke Koba‡

Anne E. Giblin†

Marissa S. Weiss§

Gretchen M. Gettel§ and

Gaius R. Shaver†

*Corresponding author. Center for Global Environmental Research, National Institute for Environmental Studies, Onogawa 16-2, Tsukuba 305-8506, Japan.

Present address: Rakuno Gakuen University, Midorimachi 582, Bunkyo-dai, Ebetsu 069-8501, Japan. shobara@rakuno.ac.jp

†The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA 02543, U.S.A.

‡Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of Technology, Yokohama 226-8502, Japan.

§Department of Ecology and Evolutionary Biology, Cornell University, NY 14853, U.S.A.

Abstract

Atmospheric nitrogen (N) fixation is a key N input to arctic ecosystems, but relatively few estimates of annual N-fixation rates are available. We measured N-fixation of plant-soil cores by the acetylene reduction technique at different topographic positions in an upland tundra watershed, Imnavait Creek, through two growing seasons in order to evaluate spatial and temporal variation in N-fixation. We also examined the effects of light and temperature on N-fixation to estimate annual N-fixation rates of surface soil in this watershed using field meteorological data. Surface soil at Imnavait Creek had significant acetylene reduction potential throughout the watershed (generally 6 to 10 $\mu\text{mol C}_2\text{H}_4 \text{ m}^{-2} \text{ h}^{-1}$), indicating that N-fixing organisms were present everywhere. Although acetylene reduction potential was roughly constant through the growing season, moisture, temperature and light intensity strongly affected the measured acetylene reduction rates in laboratory incubations. In addition, the relatively few samples that included the lichen, *Peltigera aphosa*, had significantly greater acetylene reduction potential, although the overall influence of *Peltigera* on N-fixation in this watershed seems to be small. The N input via N-fixation at Imnavait Creek was estimated at 80 to 131 $\text{mg N m}^{-2} \text{ yr}^{-1}$, indicating that N-fixation contributed 85 to 90% of total watershed N inputs.

Introduction

Tundra and boreal ecosystems contain globally significant stocks of carbon (C) (Bliss and Matveyeva, 1992; Oechel et al., 1993), and the accumulation and cycling of this C is tightly linked to the availability and turnover of other elements, particularly N (Shaver et al., 1992; McKane et al., 1997a, 1997b; Jonasson et al., 2001). Because the C and N cycles are often tightly linked in these northern ecosystems, one of the keys to understanding of northern C cycling is improved understanding of N inputs, outputs, and turnover. Furthermore, because several recent reports have predicted major increases in temperature in this century in the polar regions (e.g., Serreze et al., 2000; Cubasch et al., 2001), understanding the effects of changes in temperature and other climate variables on N cycling is essential to prediction of change in northern C cycles.

Atmospheric N fixation is a key N input to arctic ecosystems (Bazely and Jefferies, 1989; Chapin and Bledsoe, 1992), although previously measured rates are very low in comparison with lower latitudes (at most 300 $\text{mg m}^{-2} \text{ yr}^{-1}$; Alexander and Schell, 1973; Barsdate and Alexander, 1975; Van Cleve and Alexander, 1981; Chapin et al., 1991; Chapin and Bledsoe, 1992). Because N deposition in the Arctic is very low, ranging from 8 to 56 $\text{mg m}^{-2} \text{ yr}^{-1}$ (Van Cleve and Alexander, 1981; Arctic LTER database), accurate estimation of N-fixation rates may be of importance for understanding of arctic N cycling. However, the contribution of N-fixation to ecosystem N inputs has just begun to be examined especially for upland tundra ecosystems

(Weiss, 2003), in spite of their extensive distribution in the Arctic (Bliss and Matveyeva, 1992).

Free-living cyanobacteria are the dominant N-fixing organisms in the Arctic (Alexander and Schell, 1973; Alexander et al., 1978), particularly the widespread genus *Nostoc* which can live on both plants and the soil surface (Chapin and Bledsoe, 1992). Nitrogenase activity in *Nostoc* is highly responsive to environmental factors such as temperature, light, and moisture (Alexander et al., 1978; Chapin and Bledsoe, 1992), and these factors often lead to spatial and temporal variability in N-fixation rate (Basilier et al., 1978; Chapin et al., 1991; Dickson, 2000). However, relatively little effort has been directed to estimation of total annual N-fixation inputs in tundra ecosystems, especially using field meteorological data and comparison of N-fixation relative to other ecosystem N inputs (Boring et al., 1988; Cleveland et al., 1999).

The objectives of this study were to characterize N-fixation of surface soil in an upland tundra toposquence, to estimate the annual N input via N-fixation at a watershed level using field meteorological data, and to clarify the contribution of N-fixation to total N input of the watershed by comparison with the annual N input via atmospheric deposition. We measured N-fixation of plant-soil cores in an upland tundra watershed, Imnavait Creek, in the northern Brooks Range, Alaska through two growing seasons (2002 and 2003) to evaluate spatial and temporal variations in N-fixation, and also examined the effects of light and temperature on N-fixation. Other components of the N budget of upland tundra have been studied intensively at Imnavait

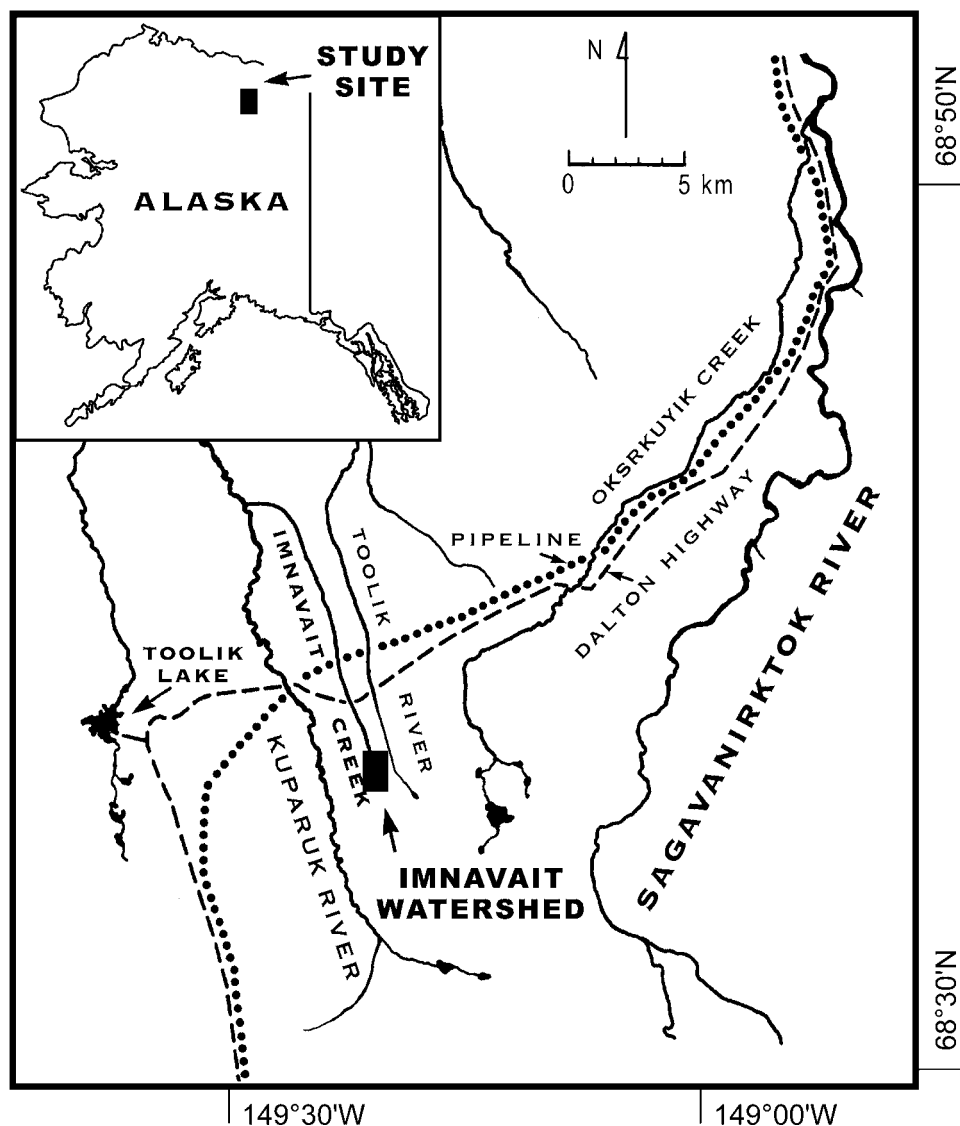


FIGURE 1. Location of the study site in arctic Alaska.

Creek and at nearby sites such as Toolik Lake and Sagavanirktok River, the sites of Arctic LTER project (e.g., Giblin et al., 1991; Shaver et al., 1991; Nadelhoffer et al., 1996; McKane et al., 2002; Hobbie and Gough, 2002; Weiss, 2003). In addition, a series of experiments have shown strong N limitation of C cycling in most nearby ecosystem types (e.g., Chapin et al., 1995; McKane et al., 1997a, 1997b; Hobbie and Chapin, 1998; Shaver et al., 1998; Johnson et al., 2000).

Study Site

Innavait Creek (68°37'N, 149°18'W; Fig. 1) is a 2.2 km² first-order watershed about 12 km east of Toolik Lake in the northern foothills of the Brooks Range in northern Alaska (Reynolds and Tenhunen, 1996). The elevation of the watershed ranges between 844 and 960 m (McNamara et al., 1998). The stream that drains the watershed runs roughly from south to north, and we conducted all sampling on the west-facing, gentle slope. This slope is underlain by permafrost and the maximum depth of soil thaw was typically 15 to 40 cm. The soils are mostly Histic Pergelic Cryaquepts (Rieger et al., 1979), of Sagavanirktok age, and range from exposed till on the ridgetop to colluvial-basin deposits on the lower slopes (Walker et al., 1989). The dominant vegetation is moist acidic tussock tundra, typical of much of the foothills region of the Brooks Range (Walker et al.,

1989), and dominated by *Eriophorum vaginatum* and *Carex bigelowii* intermixed with *Vaccinium vitis-idaea*, *Cassiope tetragona*, *Ledum palustre*, *Salix pulchra*, *Sphagnum* spp., and *Betula nana*. From 1994 through 2001 the mean annual precipitation and air temperature at Toolik Field Station averaged 322 mm and -9.0°C, respectively (Arctic LTER database; <http://ecosystems.mbl.edu/arc/default.htm>). Every year the watershed is snow covered typically from late September to late May.

In the Imnavait Creek watershed, the plant-soil cores were collected for measurement of N-fixation rates at several locations at different topographic positions because variation in the contributions of different N-fixing species among communities may also lead to variability in N input (Bowman et al., 1996). On the basis of these results and the meteorological data from this region, we estimated the annual N input by N-fixation for the watershed.

Materials and Methods

EXPERIMENTAL DESIGNS

Spatial and Temporal Patterns

To evaluate spatial and temporal variation in N-fixation, we sampled plant-soil cores throughout two growing seasons (2002 and

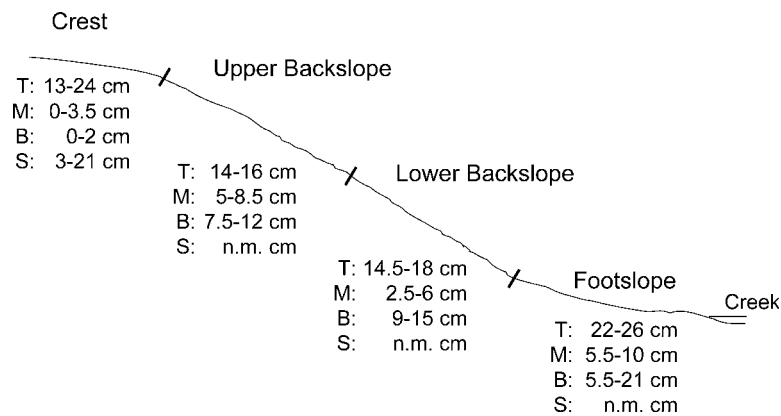


FIGURE 2. Study sites in a slope of Imnavait watershed are shown with ranges ($N=6$) of total thaw depth (T) and thickness of mossy surface organic layer (M), black organic layer (B), and mineral soil layer (S) measured on 10 July 2002. Mossy surface organic layer includes dead material of mosses. The thickness of mineral soil was not measured (indicated by n.m.) at some locations because the permafrost table was above the boundary between the organic layer and mineral soil layer at the time of measurement.

2003) within four typical sites: Crest, Upper Backslope, Lower Backslope and Footslope (Fig. 2), as characterized by Walker et al. (1989). The Crest site was located in dry heath vegetation on the ridge crest. The Upper and Lower Backslope sites were located in moist acidic tussock tundra. The Footslope site was located in wet sedge tundra at the base of the slope. Thickness of soil layers in these sites is also shown in Figure 2.

Sampling was conducted from 9 July to 7 August in 2002 and from 28 June to 5 August in 2003. At each sample date, three plant-soil cores (5.3 cm diameter) were collected at random from the upper half and from the lower half of each site, a total of six cores for each site and date. After sampling the top 3 cm of each core was removed and stored in the dark at 4°C for 14 to 16 h before incubation. Preliminary experiments indicated that there were no significant differences between tussock and inter-tussock areas.

Measurements of N-fixation were made using the acetylene reduction technique (Hardy et al., 1968). Plant-soil core samples were enclosed in 475-mL glass canning jars. The lids of the jars had been modified to hold rubber septa for injection and removal of headspace gases using syringes. We injected acetylene into the jars to yield a 10% (v/v) concentration and allowed them to incubate at 12°C and a light level of approximately 350 to 550 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (photosynthetically active photon flux density). Gas samples (5 mL) were collected with gas-tight syringes 2 h after the beginning of incubation and again 3 to 4 h later. Ethylene concentrations of the collected gasses were immediately measured on a Shimadzu gas chromatograph (GC-14A) with flame-ionization detector (FID). The change in ethylene concentration between the first and second samplings was then used to calculate rate of acetylene reduction. Preliminary assays over shorter time intervals showed constant increases in ethylene concentration from 2 to 6 h after the beginning of incubation and that the period length was sufficient to have clear peaks. Blank tests showed very low ethylene production, $0.38 \pm 0.31 \mu\text{mol C}_2\text{H}_4 \text{ m}^{-2} \text{ h}^{-1}$ in average. After incubations the cores were dried and weighed. For each core, presence or absence of the lichen *Peltigera aphthosa* was noted because we found from preliminary tests that *Peltigera* can have a superior rate of acetylene reduction compared to other major species of plants, mosses, and lichens found in this watershed. An index of *Peltigera* abundance was determined using the percentage of cores containing *Peltigera* to total cores. There are many researches reporting that lichens found in tundras can have a high rate of N-fixation (e.g., Alexander et al., 1978; Weiss, 2003).

Core Stratification

To determine N-fixation with depth in the soil, cores from the Upper Backslope site were stratified into four segments: 0–3 cm (plants and upper organic matter), 3–8 cm (middle organic matter), >8 cm (lower organic matter), and mineral soil. Acetylene reduction activity at each depth was measured by the same procedure as in the experiments on spatial and temporal patterns.

Light Response

To assess the effect of light on N-fixation, plant-soil cores were taken from the Upper Backslope on 16 July and 7 August 2002 and from all the sites on 17, 23, and 31 July 2003. These cores were first incubated in the dark for 3 to 4 h and the light level was then increased over an additional 3 to 4 h incubation. This procedure was repeated until each core had been incubated at either 6 or 12°C (typical air temperature in spring and autumn or summer around the study sites) under four to five different light levels from 0 to 1019 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Light levels were adjusted by placing one or more mesh screens (mosquito netting) between the light source and the chambers, as measured with a photosynthetically active radiation (PAR) meter. At each light level, acetylene reduction activity was estimated as ethylene accumulation over the last 2 h of incubation. In this experiment the cores including *Peltigera* were not used.

Temperature Response

For the temperature experiment plant-soil cores were sampled from the Upper Backslope on 12 August 2002 and from the Lower Backslope and Footslope on 8 August 2003. We measured the acetylene reduction rates by incubating these cores over a range of temperatures (4, 8, 17, and 30°C for 2002 and 8, 17, and 30°C for 2003) under a light level of approximately 350 to 550 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD.

Estimation of Annual Rates of N Fixation in the Watershed

The annual rates of N-fixation in the watershed were estimated using the hourly data for summertime PPFD and air temperature recorded at Toolik Field Station from 1994 to 2001 (Arctic LTER Database), about 20 km away from the study site, and using coefficients determined by the light and temperature experiments of this study. The details concerning to determining the coefficients and modeling the annual rates are described in the Results section.

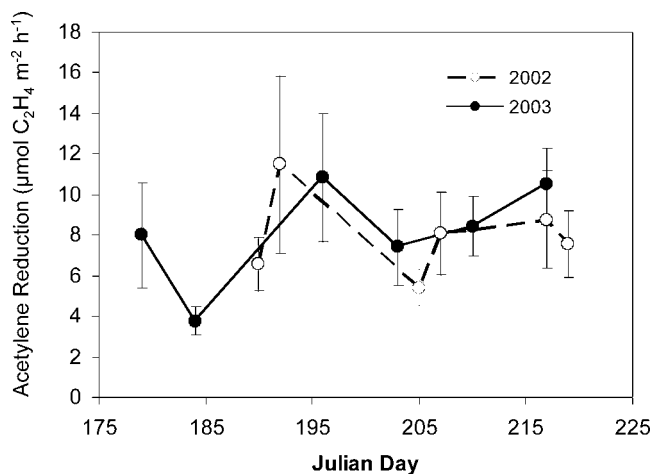


FIGURE 3. Seasonal changes in acetylene reduction potential of surface soils during the growing seasons of 2002 (open circles) and 2003 (filled circles).

Calibration of Acetylene Reduction Assay Using $^{15}\text{N}_2$

To calculate the conversion factor between acetylene reduced and N fixed, we carried out a preliminary experiment in July of 2000. Soil core samples ($n = 5-8$) were collected from each slope position in three different toposequences at Innavait Creek (total $n = 23$). Acetylene reduction rates of the top 3 cm of the cores were measured using FID-GC as described above. Immediately after the measurement, the incubation chambers were opened for several hours to allow the acetylene and ethylene to escape from the chambers. The following day, the chambers were resealed after addition of deionized water. The water volumes were set by calculating the difference in each core's weight before and after the incubation. Based on the acetylene reduction measured in the previous day, six core samples with high rates of acetylene reduction were chosen for $^{15}\text{N}_2$ -incorporation measurement because we expected that relatively low rates of acetylene reduction would make the calculation of ^{15}N incorporation difficult. Another four core samples were used to determine the difference in acetylene reduction rate between day 0 and day 1. A measured quantity of $^{15}\text{N}_2$ (ca 10% of headspace) was added to each of the six chambers before a 24-h incubation. The other four chambers were incubated for about 22 h after acetylene addition, and the acetylene reduction rates of the cores were measured as above. The acetylene reduction rates measured on day 1 ($\text{AR}_{\text{day } 1}$) were slightly lower than those measured on day 0 ($\text{AR}_{\text{day } 0}$) as follows:

$$\text{AR}_{\text{day } 1} = \text{AR}_{\text{day } 0} \times 0.575 + 0.0319$$

($\mu\text{mol/h}$ in chamber: $n = 4, R^2 = 0.94$)

Thus the acetylene reduction data required for calculation of the conversion factor were recalculated according to this relationship. After the incubation the cores were dried at 60°C for more than 48 h and weighed. The dry samples for $^{15}\text{N}_2$ -incorporation measurement were finely ground, and ^{15}N concentrations were measured using a continuous-flow mass spectrometer to calculate the amount of ^{15}N incorporated through the incubation (PDZ Europa 20/20 with ANCA-SL elemental analyzer, PDZ Europa, Cheshire, UK) in Stable Isotope Laboratory in Marine Biological Laboratory. The rate of $^{15}\text{N}_2$ incorporation was compared with the rate of acetylene reduction to calculate the conversion factor (Weaver and Danso, 1994).

Statistical Analyses

All statistical analyses of the field and experimental data were done using SPSS software (StatView 5.0J, SAS Institute). Compar-

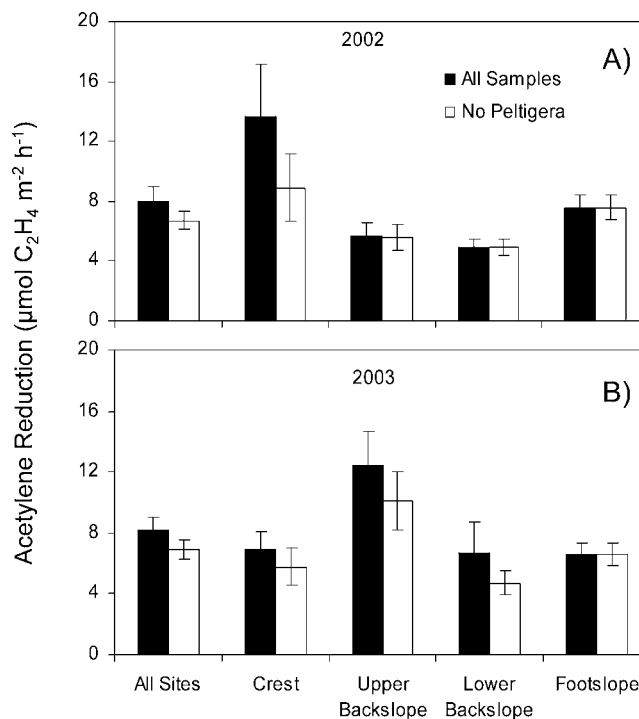


FIGURE 4. Site-to-site variation in acetylene reduction potential of surface soils in 2002 (A) and 2003 (B).

isons of mean acetylene reduction rates were done using one-way ANOVA and the Tukey post-hoc test. This method was used in comparisons for detection of differences in acetylene reduction rate among sites for each year.

Results

TEMPORAL AND SPATIAL PATTERNS

The mean acetylene reduction rates ranged generally from 6 to $10 \mu\text{mol C}_2\text{H}_4 \text{ m}^{-2} \text{ h}^{-1}$ at all sites (Fig. 3), but did not vary significantly within either growing season. There were no clear differences in acetylene reduction rates between the two growing seasons, and the overall average rates of acetylene reduction for plant-soil cores from all sites in 2002 were the same as in 2003.

In 2002, the season-long average rate of acetylene reduction for the cores including *Peltigera* was highest at the Crest site and significantly ($P < 0.05$) different from those at the Upper Backslope and Lower Backslope sites (Fig. 4A). There were no other significant differences in acetylene reduction among sites in 2002. Acetylene reduction rate in 2003 was highest at Upper Backslope but did not vary significantly among sites (Fig. 4B). Among-site differences in acetylene reduction for cores without *Peltigera* were comparable to those for all cores. The cores including *Peltigera* were more abundant in upper slope sites (Table 1) and had significantly ($P < 0.001$) higher rates of acetylene reduction compared to cores without *Peltigera* (Fig. 5).

Surface soil (0-3 cm) had the highest rate of acetylene reduction among stratified soil materials (Table 2). All the other soil depths had acetylene reduction rates near to zero and were significantly ($P < 0.001$) lower than those of surface soil (0-3 cm).

MOISTURE

Soils from lower slope position sites were wetter (Table 1). Overall, acetylene reduction of plant-soil cores in this watershed was unrelated to moisture (Fig. 6). For the plant-soil cores without

TABLE 1

Mean moisture ± 1 SE and *Peltigera* appearance of surface soil in the four study sites over the study period ($N = 65$). Letters a, b, and c in mean moisture indicates significant ($P < 0.05$) difference between sites.

Site	Moisture (%)	<i>Peltigera</i> abundance (%)
Crest	265 \pm 24 a	22.2
Upper backslope	560 \pm 36 b	12.5
Lower backslope	621 \pm 39 b	4.2
Footslope	1015 \pm 31 c	0.0

Peltigera, acetylene reduction was significantly and positively ($P < 0.001$) related to soil moisture although with a very shallow slope. At soil water content less than 750% of dry mass, many cores had insignificant acetylene reduction ($< 3 \mu\text{mol C}_2\text{H}_4 \text{ m}^{-2} \text{ h}^{-1}$), while cores including *Peltigera* often exhibited higher rates of acetylene reduction ($> 40 \mu\text{mol C}_2\text{H}_4 \text{ m}^{-2} \text{ h}^{-1}$). On the other hand, at high soil water content ($> 750\%$ of dry mass), most cores exhibited substantial rates of acetylene reduction but did not have the extremely high reduction potential of *Peltigera* cores at lower water content.

TEMPERATURE RESPONSE

Increasing temperature led to a significant increase in acetylene reduction rates of plant-soil cores (Fig. 7; $R = 0.82$, $N = 46$, $P < 0.001$). There were no significant differences in temperature response of acetylene reduction among sites. Apparently 30°C is at or below the temperature optimum, while low temperatures (4 and 8°C) still allowed some acetylene reduction to occur.

LIGHT RESPONSE

In the experiments conducted on 17 July and 7 August 2002, acetylene reduction of plant-soil cores from the Upper Backslope site increased with light intensity especially below $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD (Fig. 8). In the light experiments conducted in 2003 the cores incubated at 12°C showed a similar trend, while there were no significant increases in acetylene reduction with light intensity for the cores incubated at 6°C (Fig. 9).

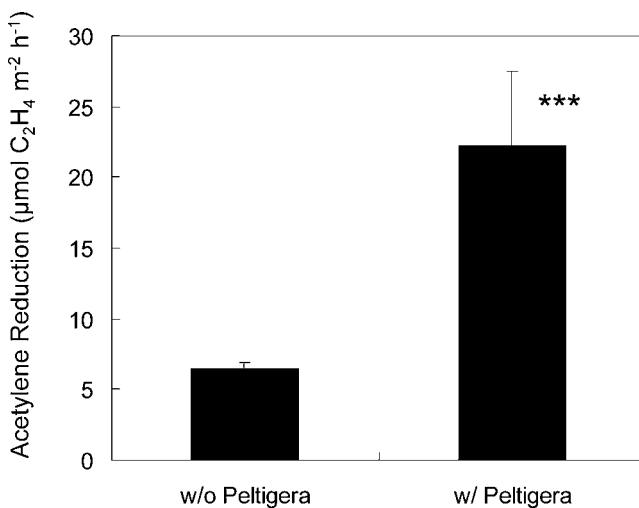


FIGURE 5. Mean acetylene reduction rate for samples with and without *Peltigera aphosa*. *** indicates difference significant at $P < 0.001$.

TABLE 2

Mean acetylene reduction rates and standard errors ($\mu\text{mol C}_2\text{H}_4 \text{ m}^{-2} \text{ h}^{-1}$) in organic materials (0–3, 3–8, and > 8 cm sections) and mineral soil. Letters a, b, and c in average indicates that the average is significantly ($P < 0.05$) different between sections.

Section	$\mu\text{mol C}_2\text{H}_4 \text{ m}^{-2} \text{ h}^{-1}$	
	Average	SE
Upper organic (0–3 cm)	7.10a	1.71
Middle organic (3–8 cm)	–0.84b	0.12
Lower organic (> 8 cm)	–0.73b	0.09
Mineral soil	–1.06b	0.30

CONVERSION FACTOR

The acetylene reduction rates measured in July of 2000 ranged from 0.87 to $75.1 \mu\text{mol m}^{-2} \text{ h}^{-1}$ (mean \pm S.D. = 20.6 ± 0.8 , $n = 23$). There was a significant correlation between acetylene reduction and the ^{15}N incorporation rate ($R^2 = 0.99$, $n = 6$) and the calculated conversion factor was 4.9 ± 1.3 (mean \pm S.D., $n = 6$). This value is slightly higher than the theoretical value (3–4) and the conversion factors measured for lichens *Peltigera aphosa* (2.9 ± 0.4) and *P. polydactyla* (3.5 ± 0.3) (Weiss, 2003).

MODELING AND ESTIMATION OF THE ANNUAL N-FIXATION IN THE WATERSHED

Our estimation of annual N fixation uses the meteorological data of air temperature and light intensity recorded at Toolik Field Station from 1994 to 2001. Moisture influence was thus not included in the estimates. Moisture effects, although statistically significant, were very small in magnitude in the cores without *Peltigera* and there was no significant moisture effect overall or in cores with *Peltigera* (Fig. 6). On the basis of the results from the light and temperature experiments, we made several assumptions to develop an estimate of the annual rates of N-fixation. From the light experiments (Fig. 7), we assumed the

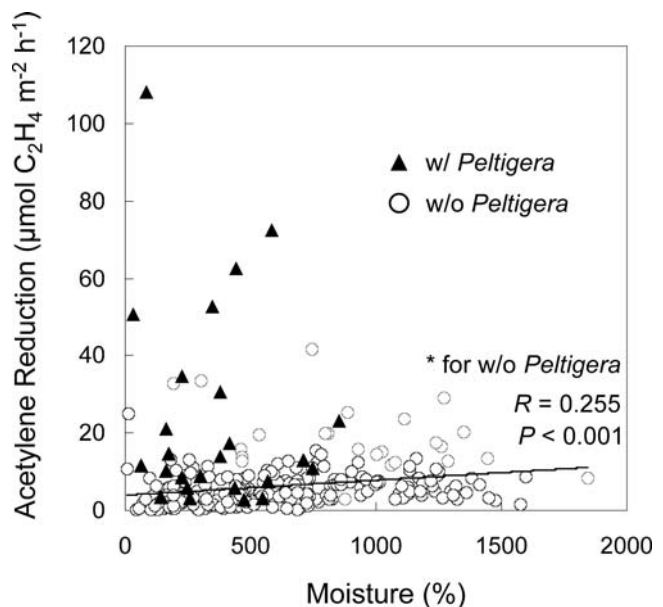


FIGURE 6. Relationship between moisture and acetylene reduction rate for samples with *Peltigera aphosa* (filled symbols) and without *Peltigera aphosa* (open symbols). Statistical analyses were done for samples with and without *Peltigera aphosa* separately.

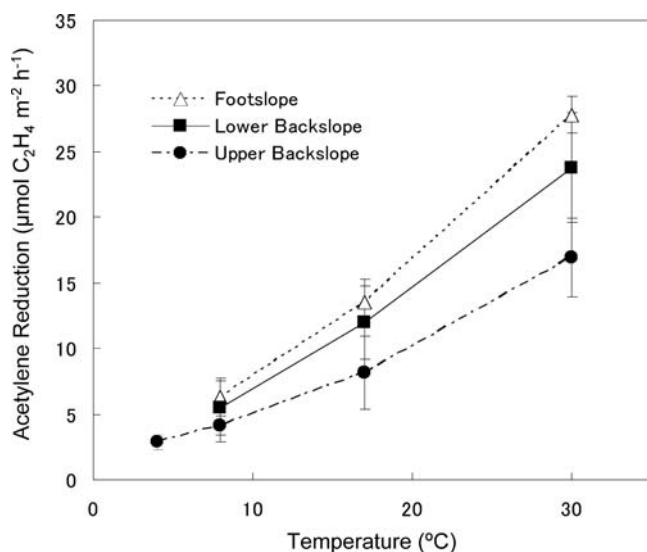


FIGURE 7. The temperature response of acetylene reduction in plant-soil cores from Footslope (open triangles), Lower Backslope (filled squares), and Upper Backslope (filled circles).

acetylene reduction activity typically increases with temperature according to the following equation:

$$f(T) = 0.804T - 1.284, \quad (1)$$

where f indicates acetylene reduction rate ($\mu\text{mol C}_2\text{H}_4 \text{ m}^{-2} \text{ h}^{-1}$) and T indicates temperature ($^{\circ}\text{C}$). The R^2 for the correlation between temperature and acetylene reduction was 0.672 and its probability (P) was less than 0.001 for any site. An exponential regression of the same data resulted in a lower R^2 value although it was also highly significant. Equation (1) was used to predict N-fixation at temperatures greater 1.6°C , and we assumed acetylene reduction is zero at temperatures below 1.6°C , while quite low temperature around 0°C can allow a bit, but detectable acetylene reduction to occur (Dickson, 2000; Zielke et al., 2002). At the low end of the temperature range ($<6-8^{\circ}\text{C}$), light intensity is unlikely to control the rate of acetylene reduction (Fig. 9). Thus we assumed that equation (1) alone could be used to estimate acetylene reduction rates at temperatures from 1.6 to 8°C . At higher temperatures, light intensity influences acetylene reduction rates (Figs. 5, 6) but the rate saturates above about $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD. Thus we assumed a linear increase in acetylene reduction with increasing

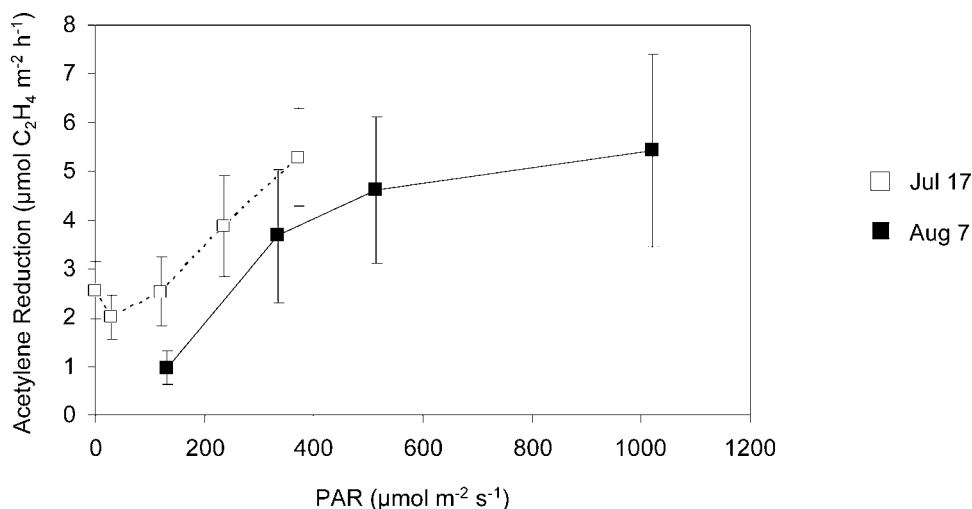


FIGURE 8. The response of acetylene reduction to varying light intensity in experiments conducted on 17 July (open symbols) and 7 August (filled symbols) 2002.

light availability until light intensity reaches this level. We also assumed that temperature affects the slope of the light response but does not affect the y-intercept ($3.4 \mu\text{mol C}_2\text{H}_4 \text{ m}^{-2} \text{ h}^{-1}$). The following relationship was thus used to estimate the acetylene reduction rates at temperatures greater than 8°C and under light intensities from 0 to $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$:

$$f(T, L) = \frac{(0.804T - 1.284) - 3.4}{500} L + 3.4, \quad (2)$$

where L indicates light intensity ($\mu\text{mol m}^{-2} \text{ s}^{-1}$). Above $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PPFD, temperature is the only factor controlling acetylene reduction rates, so the equation (1) was used. Conversion of acetylene reduction values to N-fixation rates was made using three different conversion factors 3, 4 (theoretical values), and 4.9 (this study). The theoretical conversion factor between ethylene produced in the acetylene reduction experiment and N fixed in N-fixation is between 3 and 4, depending on the nitrogenase acting as an ATP-dependent hydrogenase (Jensen and Cox, 1983). However, previous experiments have observed values between <0.01 and 25 (Bergersen, 1970; Nohrstedt, 1983; Zechmeister-Boltenstern and Kinzel, 1990; Liengen, 1999), indicating a possibly wider range.

The mean monthly rates of N-fixation estimated using a conversion factor of 4.9 for 1994–2001 are shown in Figure 10. N fixation occurred through the entire growing season, from May to September; it was greatest in July and insignificant or zero in the other months. Monthly N-fixation rate during the growing season ranged from 5.8 to $28.1 \text{ mg N m}^{-2} \text{ mo}^{-1}$. Annual N-fixation rate was slightly different among years, averaging 131, 98, and $80 \text{ mg N m}^{-2} \text{ yr}^{-1}$ for conversion factors 3, 4, and 4.9, respectively (Table 3). The rates of N-fixation in the Innvait watershed were approximately 6 to 10 times higher than N deposition, and the contribution of N-fixation to total watershed N input averaged to 90, 87, and 85% for conversion factors 3, 4, and 4.9, respectively.

Discussion

Plant-soil cores from the soil surface in all communities sampled at the Innvait watershed exhibited substantial acetylene reduction potential, averaging $\sim 8 \mu\text{mol C}_2\text{H}_4 \text{ m}^{-2} \text{ h}^{-1}$. Similar acetylene reduction activity in surface soil has been reported from other arctic sites (Alexander et al., 1978; Chapin et al., 1991; Dickson, 2000; Zielke et al., 2002). Cyanobacteria including the genus *Nostoc* are the predominant N-fixers and are common in surface soils throughout the Arctic (Alexander et al., 1978; Chapin and Bledsoe, 1992). Many

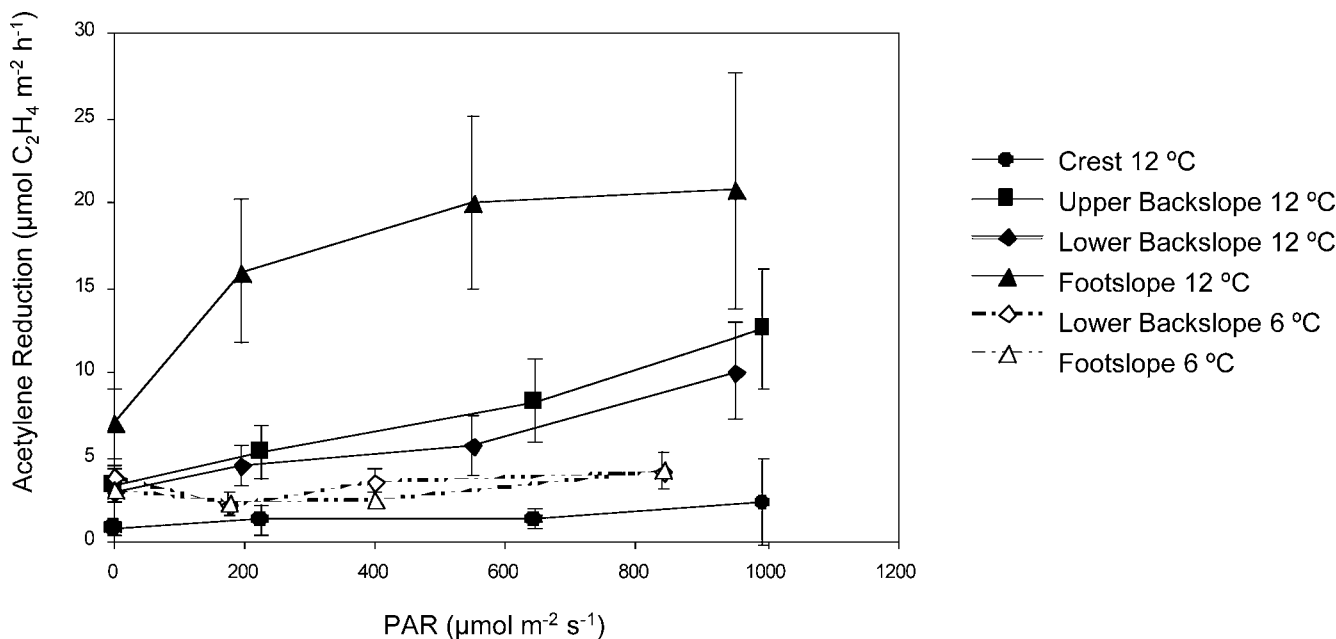


FIGURE 9. The response of acetylene reduction to varying light intensity in experiments conducted in 2003. Filled and open symbols indicate incubation at 12° and 6°C, respectively.

lichen and moss species of the Arctic and Subarctic have also been reported to fix N through associations with cyanobacteria (Alexander et al., 1978; Chapin and Bledsoe, 1992; DeLuca, 2001). The high rates of acetylene reduction in cores including the lichen *Peltigera aphosa* (Figs. 5, 6) from our study sites are similar to those reported for *P. aphosa* from nearby the Toolik Lake (Weiss, 2003), while the contribution of the lichen to the overall rate of N-fixation in our study sites was low (Fig. 4) presumably due to its low abundance. It is possible that other host plants containing N-fixing symbionts at our study sites (particularly mosses) also contributed to the acetylene reduction activity that we measured although we did not attempt to distinguish other species' contributions to the overall rates.

The season-long monitoring of acetylene reduction in this study shows that the seasonal and site-to-site differences in acetylene reduction potential were small or insignificant for both growing seasons (Fig. 3). Others have reported stronger spatial and temporal variation at other arctic sites (Alexander et al., 1978; Chapin and Bledsoe, 1992). Why was acetylene reduction potential so invariable in our study? In the following section, we discuss the controls of

environmental factors such as moisture, temperature, light intensity and distribution of N-fixing organisms on the variability of acetylene reduction rates in surface soil.

MOISTURE

Other studies have concluded that moisture is a major environmental factor controlling N-fixation rate in the Arctic (Alexander, 1974; Chapin and Bledsoe, 1992). In many cases, seasonal changes and spatial differences in acetylene reduction rate obtained from incubation under uniform environmental conditions can be ascribed to the *in situ* moisture regime of the sample (Alexander et al., 1978; Chapin et al., 1991; Dickson, 2000). In our study, the overall site-to-site variations in moisture did not reflect those of acetylene reduction rate, while the correlation between moisture and acetylene reduction for individual cores without *Peltigera* provides some evidence for the importance of moisture as a regulator N-fixation in the field. The reason for the lack of an overall correlation can be explained as follows: moisture typically controlled the acetylene reduction rate but the control was counterbalanced

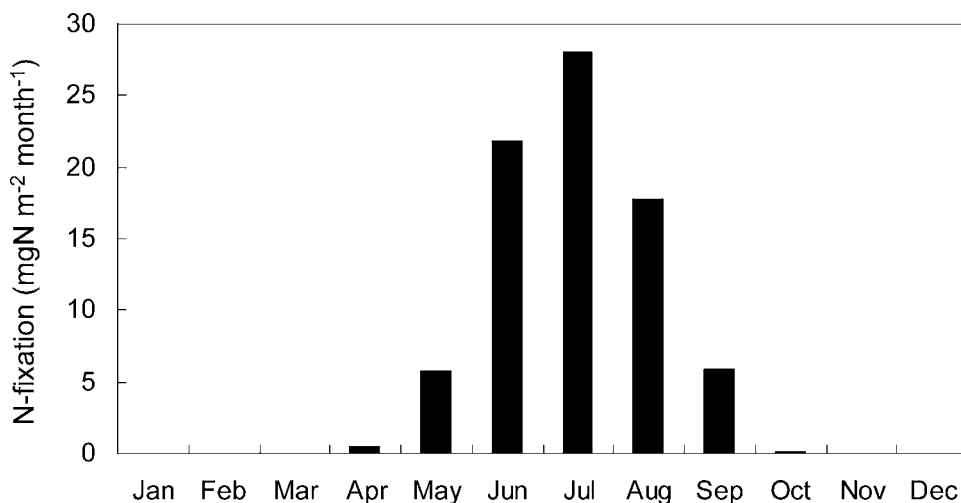


FIGURE 10. Predicted monthly N-fixation rates of surface soil in the Innavait watershed using a conversion factor of 4.9. Values are means calculated using weather data from 1994 to 2001.

TABLE 3

Estimated annual rate of N-fixation in the Imnavait Watershed, N deposition at Toolik Lake, and contribution of N-fixation to total N-inputs in variable conversion factor (CF).

Year	N inputs (mgN m ⁻² yr ⁻¹)				Contribution of N-fixation to total N inputs (%)		
	N fixation			N deposition*	CF = 3	CF = 4	CF = 4.9
	CF = 3	CF = 4	CF = 4.9				
1994	133	100	81	8	94	93	91
1995	134	100	82	14	91	88	85
1996	111	83	68	10	92	89	87
1997	146	110	89	15	91	88	86
1998	154	116	95	15	91	89	86
1999	145	108	89	19	88	85	82
2000	109	81	67	14	89	85	83
2001	115	86	70	20	85	81	78
Average	131	98	80	14	90	87	85

* Inorganic N in unfrozen precipitation during growing season (May to August) (Arctic LTER database).

by the relatively strong acetylene reduction of N-fixers such as *Peltigera* which were more abundant in drier sites in the watershed (Table 1).

TEMPERATURE

Acetylene reduction rate increased with temperature from 4 to 30°C and showed no sign of an optimum or maximum below 30°C (Fig. 7). The temperature response was similar to that of *Peltigera apthosa* collected at Toolik Lake (Weiss, 2003) and to other arctic soils (Dickson, 2000; Zielke et al., 2002), although the optimum temperature for acetylene reduction of surface soil is typically below 25°C (Alexander, 1974; Alexander et al., 1978; Chapin et al., 1991; Chapin and Bledsoe, 1992). The season-long measurements of acetylene reduction obtained from incubation under uniform temperature conditions (12°C) showed no significant change during the growing season. However, in the field with varying temperature, N-fixation would have exhibited distinct temporal (diurnal and seasonal) variation, as simulated N-fixation rate shows (Fig. 10).

LIGHT

Although in the experiment conducted at 12°C acetylene reduction rate increased with light intensity and was saturated or constant at high light intensity (Figs. 8, 9), in the 6°C treatment the light response of surface soil was small or insignificant. Light-dependence of acetylene reduction has been found in *Nostoc* (Alexander et al., 1978; Basilier et al., 1978), *Peltigera* (Alexander et al., 1978; Weiss, 2003) and soil surface in the Arctic (Chapin et al., 1991), and the light response of acetylene reduction often changes with temperature (Alexander et al., 1978; Weiss, 2003). Especially at the low temperatures common in surface soils at Imnavait Creek (~3–5°C), the light response of acetylene reduction is very small (Alexander et al., 1978), which is consistent with our result.

The plant-soil core samples in our study were stored in dark and cool conditions for one night before the experiments, yet we still found substantial reduction of acetylene in the initial measurements at 0 μmol m⁻² s⁻¹ PPFD (Figs. 8, 9). This suggests that substantial amounts of stored energy are available for free living N-fixers in the litter, wood, dead roots, or in the mineral soil (Chapin and Bledsoe, 1992).

CONTRIBUTION OF N-FIXATION TO WATERSHED N INPUTS

Ecosystem N input as N-fixation in the Arctic ranges from 6 to 300 mg N m⁻² yr⁻¹, and for most sites is between 10 and 120 mg N

m⁻² yr⁻¹ (Alexander and Schell, 1973; Barsdate and Alexander, 1975; Kay and Virginia, 1989; Chapin and Bledsoe, 1992; Cleveland et al., 1999). Our estimated values were within this range (Table 3), while they are considerably low compared to the annual N uptake requirement for upland tundra on the North Slope of Alaska (1–3 g N m⁻² yr⁻¹; Shaver and Chapin, 1991; Leadley et al., 1996). The N-fixation rates in circumpolar regions are generally very small compared to those in temperate and tropical forests (Cleveland et al., 1999), reflecting the strong influence of temperature and light (or length of growing season) on nitrogenase activity.

The contribution of N-fixation to total N inputs in the Imnavait watershed was high compared to atmospheric N deposition and in the upper range of values from other arctic sites (25 to 82% of total N input; Chapin and Bledsoe, 1992). However, the annual N input as N deposition cited in Table 3 lacks N deposition during the dormant (winter) season. Shaver et al. (1991) reported that the annual wintertime N deposition in the nearby Sagavanirktok River valley was 10 to 20 mg N m⁻² yr⁻¹, depending mainly on snow depth and snow water content, which were comparable to the growing season N deposition. Recalculated including these data, the contribution of N-fixation to total N inputs in the watershed average 79–84%, 74–80%, and 70–77% for conversion factors 3, 4, and 4.9, respectively. This still indicates that N-fixation is the dominant atmospheric N input in the watershed.

The importance of N-fixation in terrestrial ecosystems could vary widely depending upon the presence of species that harbor symbiotic bacteria (Boring et al., 1988; Schlesinger, 1997). Globally, the usual ranges of N inputs in nonagricultural terrestrial ecosystems are 100–1200 mg N m⁻² yr⁻¹ from atmospheric deposition and 100–500 mg N m⁻² yr⁻¹ from nonsymbiotic biological fixation, while symbiotic fixation during early succession stages generally ranges from 1000–16,000 mg N m⁻² yr⁻¹ (Boring et al., 1988). Although the influence of *Peltigera* on N-fixation in this watershed was relatively small because of its low abundance, we found that plant-soil cores including *Peltigera* often exhibited considerably greater acetylene reduction rates than those without *Peltigera*. Weiss (2003) reported that N-fixation rate by lichens at upland tundras around Toolik Lake amounts to 1–23 mg N m⁻² yr⁻¹. It is therefore possible that higher (<+50%) annual rates of N-fixation than we estimated may prevail in Alaskan upland tundras with higher lichen abundance.

It has been predicted that arctic air temperatures will increase by 1.4 to 5.8°C during the 21st century (Cubasch et al., 2001). If we recalculate our annual prediction of N-fixation using +3°C increased air temperatures, the annual rate of N-fixation increases 34% on average from 1994 to 2001. Due to the strong response of N-fixation to air temperature, such a temperature increase could lead to substantially increased N-fixation if the presence and composition of species which harbor symbiotic bacteria in the ecosystem does not change.

Conclusion

Surface soil throughout the watershed at Imnavait Creek has significant acetylene reduction potential, indicating the ubiquitous presence of N-fixing organisms. Although acetylene reduction potential was roughly constant through two growing seasons, moisture, temperature, and light intensity also affected the acetylene reduction rates in the field. In addition, abundance and distribution of N-fixing symbionts such as lichens can affect the spatial pattern of N-fixation. The input of N via N-fixation at Imnavait Creek was estimated to be several times greater than the annual N deposition rate, suggesting that N-fixation is the primary N-input process in this strongly N-limited ecosystem.

Acknowledgments

We thank Alex Breslav, Ed Rastetter, Sarah Hobbie, George Kling, Loretta Johnson, Martin Sommerkorn, Erica Stieve, Jim Laundre, Yuriko Yano, Knute Nadelhoffer, and colleagues at Toolik Field Station for their cooperation and helpful comments. We also thank the editors and anonymous reviewers for critical comments on the manuscript. This study was supported by grants from the US National Science Foundation to the Marine Biological Laboratory.

References Cited

- Alexander, V. M., Billington, M., and Schell, D. M., 1978: Nitrogen fixation in arctic and alpine tundra. In Tieszen, L. L. (ed.), *Vegetation and Production Ecology of an Alaskan Arctic Tundra*. Ecological Studies Series Vol. 29. New York: Springer-Verlag, 539–558.
- Alexander, V. M., and Schell, D. M., 1973: Seasonal and spatial variation of nitrogen fixation in the Barrow, Alaska, tundra. *Arctic and Alpine Research*, 5: 77–88.
- Arctic LTER database, 2004: http://ecosystems.mbl.edu/arc/data_doc/terrest/rain/1989_2003gsbkrm.html
- Barsdate, R. J., and Alexander, V., 1975: The nitrogen balance of arctic tundra: pathways, rates and environmental implications. *Journal of Environmental Quality*, 4: 111–117.
- Basilier, K., Granhall, U., and Stenstrom, T. A., 1978: Nitrogen fixation in wet minerotrophic moss communities of a subarctic mire. *Oikos*, 31: 236–246.
- Bazely, D. R., and Jefferies, R. L., 1989: Lesser Snow Geese and the nitrogen economy of a grazed salt-marsh. *Journal of Ecology*, 77: 24–34.
- Bergersen, F. J., 1970: The quantitative relationship between nitrogen fixation and the acetylene-reduction assay. *Australian Journal of Biological Sciences*, 23: 1015–1025.
- Bliss, L. C., and Matveyeva, N. V., 1992: Circumpolar arctic vegetation. In Chapin, F. S., III, Jefferies, R. L., Reynolds, J. F., Shaver, G. R., and Svoboda, J. (eds.), *Arctic Ecosystems in a Changing Climate: An Ecophysiological Perspective*. San Diego: Academic Press, 59–89.
- Boring, L. R., Swank, W. T., Waide, J. B., and Henderson, G. S., 1988: Sources, fates, and impacts of nitrogen inputs to terrestrial ecosystem: review and synthesis. *Biogeochemistry*, 6: 119–159.
- Bowman, W. D., Schardt, J. C., and Schmidt, S. K., 1996: Symbiotic N₂-fixation in alpine tundra: ecosystem input and variation in fixation rates among communities. *Oecologia*, 108: 345–350.
- Chapin, D. M., and Bledsoe, C., 1992: Nitrogen fixation in arctic plant communities. In Chapin, F. S., III, Jefferies, R. L., Reynolds, J. F., Shaver, G. R., and Svoboda, J. (eds.), *Arctic Ecosystems in a Changing Climate: An Ecophysiological Perspective*. San Diego: Academic Press, 301–319.
- Chapin, D. M., Bliss, L. C., and Bledsoe, L. J., 1991: Environmental regulation of nitrogen fixation in a high arctic lowland ecosystem. *Canadian Journal of Botany*, 69: 2744–2755.
- Chapin, F. S., III, Shaver, G. R., Giblin, A. E., Nadelhoffer, K. J., and Laundre, J. A., 1995: Responses of arctic tundra to experimental and observed changes in climate. *Ecology*, 76: 694–711.
- Cleveland, C. C., Townsend, A. R., Schimel, D. S., Fisher, H., Howarth, R. W., Hedin, L. O., Perakis, S. S., Latty, E. F., Von Fischer, J. C., Elseroad, A., and Wasson, M. F., 1999: Global patterns of terrestrial biological nitrogen (N₂) fixation in natural ecosystems. *Global Biogeochemical Cycles*, 13: 623–645.
- Cubasch, U., Meehl, G. A., Boer, G. J., Stouffer, R. J., Dix, M., Noda, A., Senior, C. A., Raper, S., and Yap, K. S., et al., 2001: Projections of future climate change. In: Houghton, J. T., et al. (eds.), *Climate Change: The Scientific Basis—The Third Assessment Report of Intergovernmental Panel on Climate Change (IPCC)*. Cambridge: Cambridge University Press, 525–582.
- DeLuca, T. H., Zackrisson, O., Nilsson, M. C., and Sellstedt, A., 2001: Quantifying nitrogen-fixation in feather moss carpets of boreal forests. *Nature*, 419: 917–920.
- Dickson, L. G., 2000: Constraints to nitrogen fixation by cryptogamic crusts in a polar desert ecosystem, Devon Island, N.W.T., Canada. *Arctic, Antarctic, and Alpine Research*, 32: 40–45.
- Giblin, A. E., Nadelhoffer, K. J., Shaver, G. R., Laundre, J. A., and McKerrow, A. J., 1991: Biogeochemical diversity along a riverside toposequence in arctic Alaska. *Ecological Monographs*, 61: 415–436.
- Hardy, R. W. F., Holsteinm, R. D., Jackson, E. K., and Burns, R. C., 1968: The acetylene-ethylene assay for N fixation: laboratory and field evaluation. *Plant Physiology*, 43: 1185–1207.
- Hobbie, S. E., and Gough, L., 2002: Foliar and soil nutrients in tundra on glacial landscapes of contrasting ages in northern Alaska. *Oecologia*, 131: 453–462.
- Jensen, B. B., and Cox, R. P., 1983: Direct measurements of steady-state kinetics of cyanobacterial N₂ uptake by membrane-leak mass spectrometry and comparisons between nitrogen fixation and acetylene reduction. *Applied Environmental Microbiology*, 45: 1331–1337.
- Johnson, L. C., Shaver, G. R., Cades, D. H., Rastetter, R. B., Nadelhoffer, K. J., Giblin, A. E., Laundre, J. A., and Stanley, A., 2000: Plant carbon-nutrient interactions control CO₂ exchange in Alaskan wet sedge tundra ecosystems. *Ecology*, 81: 453–469.
- Leadley, P. W., Li, H., Ostendorf, B., and Reynolds, J. F., 1996: Road-related disturbances in an arctic watershed: Analyses by a spatially explicit model of vegetation and ecosystem processes. In Reynolds, J. F., and Tenhunen, J. D. (eds.), *Landscape Function and Disturbance in Arctic Tundra*. Ecological Studies Series Vol. 120. New York: Springer-Verlag, 387–417.
- Liengen, T., 1999: Conversion factor between acetylene reduction and nitrogen fixation in free-living cyanobacteria from high arctic habitats. *Canadian Journal of Microbiology*, 45: 223–229.
- McKane, R. B., Rastetter, E. B., Shaver, G. R., Nadelhoffer, K. J., Giblin, A. E., Laundre, J. A., and Chapin, F. S., III, 1997a: Climatic effects on tundra carbon storage inferred from experimental data and a model. *Ecology*, 78: 1170–1187.
- McKane, R. B., Rastetter, E. B., Shaver, G. R., Nadelhoffer, K. J., Giblin, A. E., Laundre, J. A., and Chapin, F. S., III, 1997b: Reconstruction and analysis of historical changes in carbon storage in arctic tundra. *Ecology*, 78: 1188–1198.
- McKane, R. B., Johnson, L. C., Shaver, G. R., Nadelhoffer, K. J., Rastetter, R. B., Fry, B., Giblin, A. E., Kielland, K., Kwiatkowski, B. L., Laundre, J. A., and Murray, G., 2002: Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature*, 415: 68–71.
- McNamara, J. P., Kane, D. L., and Hinzman, L. D., 1998: An analysis of streamflow hydrology in the Kuparuk River Basin, Arctic Alaska: a nested watershed approach. *Journal of Hydrology*, 206: 39–57.
- Nadelhoffer, K. J., Shaver, G. R., Fry, B., Johnson, L. C., and McKane, R. B., 1996: ¹⁵N natural abundances and N use by tundra plants. *Oecologia*, 107: 386–394.
- Nohrstedt, H. Ö., 1983: Conversion factor between acetylene reduction and nitrogen fixation in soil: effect of water content and nitrogenase activity. *Soil Biology and Biochemistry*, 15: 275–279.
- Oechel, W. C., Hastings, S. J., Vourlitis, G. L., Jenkins, M., Reichers, G., and Grulke, N., 1993: Recent change of arctic tundra ecosystems from a net carbon sink to a source. *Nature*, 361: 520–523.
- Reynolds, J. F., and Tenhunen, J. D., 1996: *Landscape Function and Disturbance in Arctic Tundra*. Ecological Studies Vol. 83. New York: Springer-Verlag, 437 pp.
- Rieger, S., Schoephorster, D. B., and Furbush, C. E., 1979: *Exploratory Soil Survey of Alaska*. U.S. Department of Agriculture, Soil Conservation Service, Washington, D. C.
- Schlesinger, W. H., 1997: *Biogeochemistry: An Analysis of Global Change*. 2nd ed. San Diego: Academic Press, 588 pp.
- Serreze, M. C., Walsh, J. E., and Chapin, F. S., III, 2000: Observational evident of recent change in the northern high-latitude environment. *Climatic Change*, 46: 159–207.
- Shaver, G. R., and Chapin, F. S., III, 1991: Production/biomass relationships and element cycling in contrasting arctic vegetation types. *Ecological Monographs*, 61: 662–675.
- Shaver, G. R., Nadelhoffer, K. J., and Giblin, A. E., 1991: Biogeochemical diversity and element transport in a heterogenous

- landscape, the north slope of Alaska. In Turner, M. G., and Gardner, R. H. (eds.), *Quantitative Methods in Landscape Ecology: The Analysis and Interpretation of Landscape Heterogeneity*. Ecological Studies Series Vol. 82. Springer-Verlag New York, 105–125.
- Shaver, G. R., Billings, W. D., Chapin III, F. S., Giblin, A. E., Nadelhoffer, K. J., Oechel, W. C., and Rastetter, R. B., 1992: Global change and the carbon balance of arctic ecosystems. *BioScience*, 42: 433–441.
- Shaver, G. R., Johnson, L. C., Cades, D. H., Murray, G., Laundre, J. A., Rastetter, R. B., Nadelhoffer, K. J., and Giblin, A. E., 1998: Biomass accumulation and CO₂ flux in three Alaskan wet sedge tundras: Responses to nutrients, temperature, and light. *Ecological Monographs*, 68: 75–99.
- Van Cleve, K., and Alexander, V., 1981: Nitrogen cycling in tundra and boreal ecosystems. In Clark, F. E., and Rosswall, T. (eds.), *Terrestrial Nitrogen Cycles*. Ecological Bulletins Vol. 33. Stockholm: Swedish Natural Science Research Council, 375–404.
- Walker, D. A., Bainnain, E., Evans, B. M., Lederer, N. D., Nordstrand, E., and Petersen, G. W., 1989: Terrain, vegetation, and landscape evolution of the R4D research site, Brooks Range Foothills, Alaska. *Holarctic Ecology*, 12: 238–261.
- Weaver, R. W., and Danso, S. K. A., 1994: Dinitrogen Fixation. In Weaver, R. W., et al. (eds.), *Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties*. SSSA Book Series, no. 5. Madison, WI: Soil Science Society of America.
- Weiss, M. S., 2003: The contribution and environmental control of nitrogen fixation by lichens in upland arctic tundra. M.S. thesis, The University of Minnesota.
- Zechmeister-Boltenstern, S., and Kinzel, H., 1990: Non-symbiotic nitrogen fixation associated with temperate soils in relation to soil properties and vegetation. *Soil Biology and Biochemistry*, 22: 1075–1084.
- Zielke, M., Ekker, A. S., Olsen, R. A., Spjelkavik, S., and Solheim, B., 2002: The influence of abiotic factors on biological nitrogen fixation in different types of vegetation in the high arctic, Svalbard. *Arctic, Antarctic, and Alpine Research*, 34: 293–299.

Ms accepted November 2005