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Source: Arctic, Antarctic, and Alpine Research, 43(2) : 267-278

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

URL: <https://doi.org/10.1657/1938-4246-43.2.267>

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Nitrogen Inputs by Associative Cyanobacteria across a Low Arctic Tundra Landscape

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Abstract

Available soil N is a key factor limiting plant productivity in most low arctic terrestrial ecosystems. Atmospheric N₂-fixation by cyanobacteria is often the primary source of newly fixed N in these nutrient-poor environments. We examined temporal and spatial variation in N₂-fixation by the principal cyanobacterial associations (biological soil crusts, *Sphagnum* spp. associations, and *Stereocaulon paschale*) in a wide range of ecosystems within a Canadian low arctic tundra landscape, and estimated N input via N₂-fixation over the growing season using a microclimatically driven model. Moisture and temperature were the main environmental factors influencing N₂-fixation. In general, N₂-fixation rates were largest at the height of the growing season, although each N₂-fixing association had distinct seasonal patterns due to ecosystem differences in microclimatic conditions. Ecosystem types differed strongly in N₂-fixation rates with the highest N input (10.89 kg ha⁻¹ yr⁻¹) occurring in low-lying Wet Sedge Meadow and the lowest N input (0.73 kg ha⁻¹ yr⁻¹) in Xerophytic Herb Tundra on upper esker slopes. Total growing season (3 June–13 September) N₂-fixation input from measured components across a carefully mapped landscape study area (26.7 km²) was estimated at 0.68 kg ha⁻¹ yr⁻¹, which is approximately twice the estimated average N input via wet deposition. Although biological N₂-fixation input rates were small compared to internal soil N cycling rates, our data suggest that cyanobacterial associations may play an important role in determining patterns of plant productivity across low arctic tundra landscapes.

DOI: 10.1657/1938-4246-43.2.267

Introduction

Plant productivity in many arctic regions is constrained both by low soil temperature and low soil moisture content, but during the growing season soil nitrogen (N) is believed to be the primary factor limiting plant growth (Shaver and Chapin, 1980; Nadelhoffer et al., 1992; Liengen and Olsen, 1997a; Zielke et al., 2005). Atmospheric N₂-fixation is considered the primary source of new N input to arctic terrestrial ecosystems; however, there are relatively few estimates of annual N inputs via N₂-fixation (Alexander and Schell, 1973; Schell and Alexander, 1973; Bazely and Jefferies, 1989; Gunther, 1989; Chapin and Bledsoe, 1992; Hobara et al., 2006). For example, most estimates have failed to simultaneously consider all N₂-fixing associations present, the representation of different N₂-fixers within vegetation types or ecosystem types and the extent of ecosystem types within a given landscape.

New N inputs in nutrient-poor arctic ecosystems are primarily due to atmospheric N₂-fixation by cyanobacteria (Alexander, 1974; Granhall and Lid-Torsvik, 1975; Chapin and Bledsoe, 1992; Liengen, 1999a; Solheim et al., 2006). Cyanobacteria occur in symbiotic associations with a wide variety of lichens, and as a free-living component of biological soil crusts (BSCs), which are communities composed of bacteria, cyanobacteria, algae, mosses, liverworts, fungi, and lichens. Facultative symbioses between cyanobacteria and mosses, liverworts, and hornworts are also common (Smith, 1984; Granhall and Selander, 1973; Rai et al., 2000; Turetsky, 2003).

Biological N₂-fixation inputs are determined by the abundance and diversity of these N₂-fixing associations as well as several environmental factors that control their activity. For example, seasonal variation in moisture, temperature, and light lead to large temporal variability in N₂-fixation rates (Basilier and Granhall, 1978; Chapin et al., 1991; Dickson, 2000; Solheim et al., 2006). Furthermore, biological N₂-fixation inputs may be expected to vary greatly among and within vegetation-types due to spatial heterogeneities in environmental and microclimatic conditions. Accordingly, landscape-level estimates of biological N₂-fixation inputs must account for topographical variation since it is the primary determinant of soil moisture patterns and therefore of the distribution of vegetation types (Walker, 2000) and their cyanobacterial associations.

A landscape-level understanding of the temporal and spatial variation in N₂-fixation inputs, as well as the environmental controls on N₂-fixation, has broad significance not just in understanding N cycling in low arctic ecosystems, but also in predicting the potential impacts of future climatic changes (Chapin and Bledsoe, 1992). Warmer temperatures and changes in moisture availability may directly affect N₂-fixation rates, but may also indirectly affect N inputs by altering the distribution of vegetation types and their particular cyanobacterial associations across the landscape. For example, enhanced shrub growth associated with climate warming trends in the low Arctic (Goetz et al., 2005; Sturm et al., 2001) may shade out lichens and possibly other N₂-fixing associations in mesic tundra. Evaluation of the

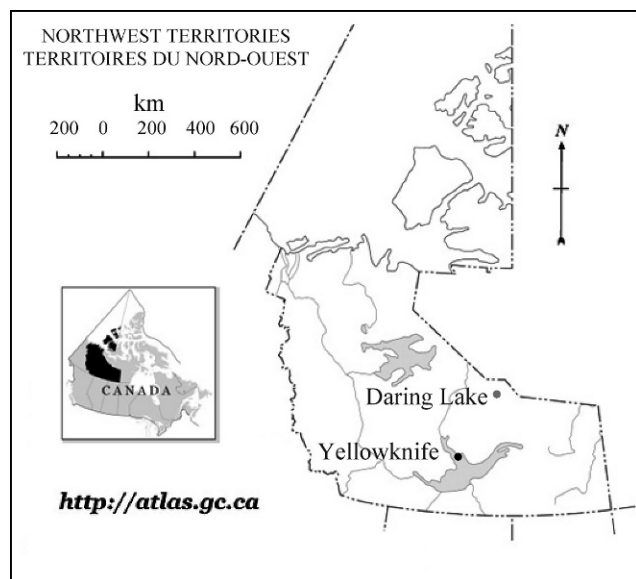


FIGURE 1. Location of the study site at Daring Lake, Northwest Territories, Canada (64°52'N, 111°35'W) (adapted from http://www.enr.gov.nt.ca/_live/pages/wpPages/Tundra_Ecosystem_Research_Station.aspx).

relative importance of these potential effects requires a spatially explicit understanding of individual ecosystem N₂-fixation rates across the landscape.

The objectives of this study were to: (a) evaluate temporal and spatial variation in N₂-fixation by associative cyanobacteria in various ecosystem types within a typical low arctic tundra landscape; and (b) estimate N input via N₂-fixation over the growing season using microclimatically driven models based on incubation studies of the ecophysiological responses of individual N₂-fixing associations to moisture, temperature, and light conditions over the growing season.

Materials and Methods

STUDY SITE

The study area was located in a low arctic tundra region at the Tundra Ecosystem Research Station, Daring Lake, Northwest Territories (64°52'N, 111°35'W, 414–470 m a.s.l.) (Fig. 1),

approximately 90 km northeast of the northern limit of continuous trees within the physiographic zone of the Bear–Slave Upland of the Canadian Shield (Obst, 2008). Landscape features include eskers, boulder fields, exposed bedrock, upland and lowland tundra, wetlands, and various sizes of lakes, ponds, and streams. Continuous permafrost is present at the site with a soil active layer ranging from 0.3 to 2 m (Obst, 2008).

Mean monthly air temperature is –30 °C in January and +13 °C in July (INAC, 2007; Obst, 2008). Mean monthly precipitation from May to October is 25 mm as rain. Snow accumulation is highly variable across the landscape, but usually ranges from 15 to 60 cm in low-lying heath vegetation by mid to late May (1996–2008; Bob Reid, INAC, unpublished data). Snowmelt usually starts after mid-May ending in early June, with some snow beds persisting on slopes until late June or early July. The plant growing season generally begins in late May or early June and ends by late August (Nobrega and Grogan, 2008; Lafleur and Humphreys, 2008).

The landscape study area encompasses the East Daring Lake Basin (26.7 km²). Ecosystem mapping and distribution of landscape units for the landscape study area follows Obst (2008). A 1-m resolution IKONOS image provided detailed information on 15 classes (plus unclassified areas) of land covers, vegetation communities, and ecosystem types present in the study area (Obst, 2008). We focused our study on the dominant ecosystem types that together occupy a total of 68% of the study area: Heath-Lichen/Heath-Mat Tundra (42%), Birch Hummock (13%), Wet Sedge Meadow (8%), and Xerophytic Herb Tundra (5%). The distribution of these four ecosystem types is largely driven by esker topography (Table 1).

N₂-FIXING ASSOCIATIONS

Four predominant cyanobacterial associations were identified within the selected landscape study area at Daring Lake: Biological Soil Crusts (BSCs) in hollows, BSCs on mineral soil mounds, *Sphagnum* spp., and *Stereocaulon paschale sensu lato*. Each cyanobacterial association was found in all of the ecosystem types included in the landscape study; however, the abundance of each association varied between ecosystem types. Vascular plant species with N₂-fixing associations, such as *Oxytropis nigrescens* (Pall.) Fisch. ex DC., and *Alnus crispa* (Aiton) Pursh., do occur in the area but are rare (Obst, 2008).

TABLE 1

The principal topographic position, substrate, drainage, and characteristic plant species for each ecosystem type included in the landscape study at Daring Lake, Northwest Territories, Canada (follows Obst, 2008).

Ecosystem type	Topographic position	Substrate/Drainage	Characteristic plant species
Xerophytic Herb Tundra	Esker tops and plateaus	Sand, gravel, rocks and boulders/ Well-drained	<i>Saxifraga tricuspidata</i> Rothb., <i>Empetrum nigrum</i> Böcher., <i>Arctostaphylos alpina</i> L., <i>Vaccinium</i> spp., Lichens
Heath-Lichen/ Heath-Mat Tundra	Esker upper sides and slopes/lower slopes and base	Sand, gravel, loam and some organic / Well-drained to moderately well-drained	<i>Betula glandulosa</i> Michx., <i>Ledum decumbens</i> Ait., <i>E. nigrum</i> , <i>Salix</i> spp., <i>A. alpina</i> , <i>Vaccinium</i> spp., Lichens
Birch Hummock	Gentle lower slopes and hummock-hollow complexes	Silts, silt loam, fine sandy loam and organic /Moderately well-drained to poorly drained	<i>B. glandulosa</i> , <i>Rubus chamaemorus</i> L., <i>Salix</i> spp., <i>L. decumbens</i> , <i>Eriophorum vaginatum</i> L., Mosses
Wet Sedge Meadow	Low-lying depressions and valley base	Well-developed organic /Saturated	<i>Carex chordorrhiza</i> Ehrh., <i>Carex rotundata</i> Wahlenb., <i>Eriophorum russeolum</i> Fr.ex Hartm., <i>Sphagnum</i> spp., <i>Salix</i> spp., <i>L. decumbens</i> .

Two major BSC communities were found in association with hummock-hollow microtopography. Hollow BSCs were found mainly in the depressions between hummocks and were composed of liverworts growing in dense mats generally underlain by varying depths of organic matter. The main components of Hollow BSCs were *Anastrophyllum minutum* Schreb. and *Cephaloziella* spp. including *C. rubella* (Nees) Warnst. and *C. hampeana* complex (O. Lee, unpublished data). Cyanobacteria on Hollow BSCs were mostly the filamentous and heterocystous cyanobacterium *Stigonema* cf. *turfaceum* (Berk.) Cooke (B. Büdel, unpublished data). However, on some samples filamentous and heterocystous *Tolypothrix* sp. and the filamentous, non-heterocystous *Schizothrix* cf. *cuspidata* W. et G.S. West, were found growing in between the leafy liverworts and on the *Stigonema* filaments. *Stigonema minutum* (C. Agardh) Hass. and *Calothrix* sp. were also found on Hollow BSC samples. Hummock BSCs were cohesive, well-developed crusts (1–2 cm thick) found on cryoturbated mineral soil mounds. Small, less well-developed patches of Hummock BSCs also occurred in sandy well-drained areas on ridge tops. Hummock BSCs were complex communities made up of lichens, mosses, and liverworts. Lichen species included *Placynthiella uliginosa* Schrader., *Bryocaulon divergens* Ach., *Bryoria tenuis* (E. Dahl) Brodo & D. Hawksw., *Cladonia* spp., *Japewia tornoensis* Nyl., *Ochrolechia frigida* Sw., and *Solorina crocea* L. (C. Bjork, unpublished data). Moss species (*Funaria* sp., *Pohlia* sp., *Ditrichum* sp., and *Polytrichum piliferum* Hedwig.) and liverwort species (*Cephalozia* sp., *Cephaloziella* sp., *Anastrophyllum* sp., *Anthelia* sp., *Lophozia* sp., and *Lophozia incise* Schrad.) were also key components of these diverse communities. *Stigonema turfaceum*, *S. minutum*, and *S. hormoides* (Kutz.) Born. & Flah. dominated Hummock BSCs; however, *Gloeocapsa decorticans* A. Braun., *G. novacekii* (Komárek & Anagnostid), *S. cuspidata*, *Anabaena* sp., and *Chroococidiopsis* sp. were also present.

Sphagnum spp. were the dominant ground cover in Wet Sedge Meadows and were found scattered in damp depressions throughout the landscape. The majority of *Sphagnum* spp. samples were composed of *Sphagnum aongstroemii* C. Hartm. and *S. subsecundum* complex, with occasional fine strands of *S. balticum* (Russ.) C. Jens. (O. Lee, unpublished data). In addition, other moss (*Drepanocladus aduncus* (Hedw.) Warnst.) and liverwort (*Gymnocolea inflata* Huds.) species were found intermingled within *Sphagnum* spp. samples. The cyanobacteria *G. decorticans* was found in association with *Sphagnum* spp. samples (B. Büdel, unpublished data).

Stereocaulon paschale was predominantly found in small continuous mats on high/mid-slope positions, often in areas where late-lying snow patches occurred. Patchy distribution of *S. paschale* also occurred on well-drained ridge tops and in hummock-hollow complexes.

N₂-FIXATION RATES

Measurements of N₂-fixation were made using acetylene reduction assays (Stewart et al., 1967). Acetylene gas (C₂H₂) was generated on-site from CaC₂ and water, with incubations injected with 10% (v/v) acetylene. Ethylene concentrations were measured in the field with a portable gas chromatograph (SRI 8610A, Wennick Scientific Corporation, Ottawa, Ontario, Canada) fitted with a Porapak column (Alltech Canada, Guelph, Ontario, Canada) and a flame ionization detector. A Stand-Alone Hydrogen Generator (SRI H₂-50, Alltech Canada, Guelph, Ontario, Canada) provided hydrogen as the carrier gas, which

was held at a constant pressure of 26 psi. Column temperature was held at 65 °C. The gas chromatograph was calibrated for each incubation with ethylene (BOC Canada Ltd., Mississauga, Ontario, Canada; C₂H₄, 98%+) that was kept at the same temperature as incubation gas samples.

Cores of Hollow BSCs ($n = 12$), Hummock BSCs ($n = 12$), and *S. paschale* ($n = 12$) were randomly sampled for each incubation from an area of ~5 km² that was representative of the larger landscape study area. Samples were taken from multiple positions on both north- and south-facing slopes of the main east-west-oriented esker. BSC samples were trimmed to an area of 19 cm² and 0.75 cm depth such that each sample had a thin underlying soil substrate. *Stereocaulon paschale* was trimmed to an area of 19 cm² and 2 cm depth, but no underlying soil substrate was included. Samples were enclosed in 250 mL glass canning jars with modified lids containing a rubber septum. The mean headspace of ARA incubations was 235.75 mL (250 mL jar volume minus 14.25 mL sample) for Hollow and Hummock BSCs and 212 mL for *S. paschale* (250 mL jar volume minus 38 mL sample). *Sphagnum* spp. cores ($n = 12$) were sampled from an area of ~0.5 km² within the Wet Sedge Meadow ecosystem type only. Samples were trimmed to an area of 56 cm² and 6 cm depth and included both live (green) and underlying decaying stems. *Sphagnum* spp. samples were incubated in 1 L canning jars with modified lids containing rubber septa and had a mean headspace of 664 mL (1000 mL jar volume minus 336 mL sample). All *Sphagnum* spp. jars were incubated *in situ* in the Wet Sedge Meadow with the glass bottom facing up and the *Sphagnum* spp. sample level with the surrounding vegetation. For each set of incubations one sample for each N₂-fixing association was used as a control, which served as both a temperature control and a blank not injected with acetylene. Control samples did not show any natural evolution of ethylene. Contamination of generated acetylene with ethylene was monitored and corrections were made for each set of incubations, as required.

Daytime ARA incubations occurred between 10:00 and 16:00 hr (6 hours) and nighttime ARA incubations between 21:00 and 7:00 hr (10 hours). A pilot study conducted in 2007 indicated net respiration in both Hollow BSCs (108 μL L⁻¹ CO₂/hr) and Hummock BSCs (162 μL L⁻¹ CO₂/hr) under average light conditions, suggesting that CO₂ limitation was unlikely to limit N₂-fixation despite longer incubation periods. However, we injected the *S. paschale* incubations with 1% (v/v) CO₂ after 3 hours for daytime incubations and after 1 hour for night-time incubations because the lichen samples lacked an underlying soil substrate to provide a CO₂ source.

Destructive sampling was used for each incubation, with new samples of each cyanobacterial association ($n = 12$) collected per incubation. Nine consecutive sets of *in situ* incubations under ambient field conditions were conducted over a 6 day period (5 nighttime and 4 daytime) in each growing season month for each N₂-fixing association, with the exception of *Sphagnum* spp. in 2007. Incubations for Hollow BSCs and Hummock BSCs were conducted on 19–24 June, 6–11 July, and 9–13 August in 2007; and on 12–17 June, 1–6 July, and 5–10 August in 2008. *Sphagnum* spp. were incubated for a 24 hr period over 5 consecutive days on 25–29 June, 9–13 July, and 17–22 August in 2007 due to logistical constraints. In 2008, *Sphagnum* spp. were incubated in the same manner as other N₂-fixing associations on 18–23 June, 7–12 July, and 17–22 August. *Sphagnum* spp. N₂-fixation rates were not significantly different between 2007 and 2008; therefore, the difference in incubation length likely had little influence on the overall rate estimation. *Stereocaulon paschale* was incubated only in 2008 on 4–9 June, 7–12 July, and 11–16 August. Over the 2007–

2008 growing seasons a total of 571 (Hollow BSCs), 572 (Hummock BSCs), 794 (*Sphagnum* spp.), and 294 (*S. paschale*) individual samples were incubated *in situ* under ambient field conditions.

With the exception of *Sphagnum* spp., all *in situ* samples were incubated outdoors near the research station laboratory under ambient field conditions. Incubation chambers were placed in water baths and bath temperature was altered to ensure that incubation temperatures reflected ambient conditions. Photosynthetically Active Radiation (PAR), air temperature, incubation temperature, and ambient temperature of Hollow BSCs, Hummock BSCs, and *S. paschale* were monitored every 30 minutes during daytime ARA incubations. On average, the surface temperature of incubation samples were within 1.5 °C of the surface temperature of the respective N₂-fixing associations under ambient conditions. Heating of incubation chambers via solar radiation was not a concern for nighttime incubations where microclimate was monitored for the first and last hour only. Moisture of Hollow BSCs, Hummock BSCs, and *S. paschale* were determined both pre- and post-incubation to ensure that drying of specimens did not occur during the incubation period. Average loss of moisture during incubations was less than <1.8% for all N₂-fixing associations. Following incubation, all samples were weighed, air dried, and then re-weighed to determine moisture content. Moisture content of samples over the growing season was later used for modeling N₂-fixation potential.

In addition to *in situ* incubations under ambient field conditions, N₂-fixing associations were also incubated *in situ* under optimal environmental conditions (200 μmol PAR m⁻² s⁻¹, 20 °C) at the end of each set of incubations in June, July, and August 2007/2008. For each of our N₂-fixing associations we likely had several different cyanobacterial species present with varying optimal operating environments; however, an optimal temperature of ~20 °C has been demonstrated for several species/environments (Basilier and Granhall, 1978; Chapin et al., 1991; Liengen, 1999a), and light saturation was been demonstrated at ~100 μmol PAR m⁻² s⁻¹ (Coxson and Kershaw, 1983a; Chapin et al., 1991). Samples (*n* = 12) were treated in the same way as field incubations, with the exception of a 24 hr wetting pretreatment at optimal hydration levels.

N₂-fixation rates for both *in situ* incubations under ambient and optimal conditions were calculated as micromoles of ethylene reduced per hour per m² based upon the length of incubation and area of each sample (19 cm² BSCs and *S. paschale*; 56 cm² *Sphagnum* spp.). Conversion ratios determined for each N₂-fixing association (see below) were used to convert ethylene reduced to N₂ reduced. ARA values were corrected for differences in incubation jar volume, mean sample volume, and area. Different N₂-fixing associations were allowed to vary in sample depth (i.e. 0.75 cm BSCs, 2 cm *S. paschale*, 6 cm *Sphagnum* spp.) to help ensure sampling units were kept intact and that N₂-fixing surfaces were representative of the different associations under natural conditions.

¹⁵N-INCUBATIONS

Samples of each cyanobacterial association were collected from the Daring Lake landscape in August 2008 to determine conversion ratios for each of the N₂-fixing associations following the methods of Liengen (1999b). Samples were kept cool (~4 °C) and shipped to the lab at the University of Northern British Columbia. Prior to incubation samples were kept at optimal hydration in a growth chamber for 72 hours under a 17/7 hr light

(200 μmol PAR m⁻² s⁻¹)/dark cycle with temperatures at 15 °C during light hours and 5 °C during dark hours. Cores for each N₂-fixing association (*n* = 8) were similar in area (19 cm²) and depth (0.75–2 cm) to those used for field incubations.

In order to achieve detection of ¹⁵N enrichment it was determined that 48 hr laboratory incubations (200 μmol PAR m⁻² s⁻¹, 20 °C) were required. Air (10% v/v) was replaced with 10% (v/v) ¹⁵N gas (Cambridge Isotope Laboratories Inc., Andover, Massachusetts, U.S.A.; ¹⁵N₂, 98%+). To reduce the potential for CO₂ limitation due to the long incubation period, each chamber was injected with 5% (v/v) CO₂. After the 48 hr incubation samples were immediately dried at 105 °C. Dry samples were ground in a ball mill and sent for ¹⁵N and total N analysis (Stable Isotope Facilities, University of Saskatchewan, Saskatoon, Saskatchewan, Canada). Control samples (*n* = 8 for each N₂-fixing association) treated in the same manner but incubated with C₂H₂ were used to determine the natural abundance of ¹⁵N and the acetylene reduction rate. The amount of N fixed was calculated using (Liengen, 1999b, p. 224):

$$Y = \left(\frac{atom\%^{15}N_{excess}}{100} \right) \times \left(\frac{totalN_{sample} \times 10^9}{t \times 28} \right) \times \left(\frac{100}{\%^{15}N_{air}} \right), \quad (1)$$

where *Y* (nmol N·gdw⁻¹·h⁻¹) are the amounts of N₂ fixed during the experiment, *atom%* ¹⁵*N*_{excess} is the difference between *atom%* ¹⁵*N*_{sample} and *atom%* ¹⁵*N*_{control}, total N is the total amount of nitrogen in the sample (g·100 gdw⁻¹), *t* is the incubation time, 28 is the molecular weight of N₂ (g/mol), and %¹⁵*N*_{air} is the percentage of ¹⁵N out of the total amount of N gas in each incubation chamber. Conversion ratios varied among the different N₂-fixing associations (Table 2).

ESTIMATION OF LANDSCAPE LEVEL N INPUTS

Microclimatic Monitoring

Hollow and Hummock BSC microclimatic conditions were monitored in several different hollow-hummock complexes within the study landscape in 2007 (Julian days 169–257) and 2008 (Julian days 154–235). PAR was measured with quantum sensors (*n* = 2–3) (LI-190 Quantum Sensors, LI-COR, Lincoln, Nebraska, U.S.A.) installed at ground level in separate hummocks and hollows and connected to a multiplexer (AM416, Campbell Scientific Inc., Edmonton, Alberta, Canada). Soil surface temperature was monitored with fine-wire copper constantan thermocouples (*n* = 7–23) connected to a multiplexer (AM25T, Campbell Scientific Inc.). Impedance clips (*n* = 4–19) were inserted at the surface of Hollow and Hummock BSCs to monitor moisture conditions (after Coxson, 1991). All multiplexers and impedance clips were connected to a datalogger (CR23X, Campbell Scientific Inc.) and hourly means recorded. Impedance measurements were calibrated in the lab by simultaneously monitoring clip values and gravimetric moisture of Hollow BSC and Hummock BSC samples from a saturated to desiccated state. Both Hollow BSC and Hummock BSC %moisture were best explained by exponential relationships with impedance clip values (*f* = 25.55*exp(1.18*x), adjusted *R*² = 0.65; and (*f* = exp(3.65*x), adjusted *R*² = 0.75).

Sphagnum spp. temperature was monitored with a pair of copper constantan thermocouples installed at a depth of 2 cm. One thermocouple was connected to a multiplexer (AM25T, Campbell Scientific Inc.) and datalogger (21X, Campbell Scientific Inc.) recording hourly means in 2007/2008. The other thermocouple was connected to an additional datalogger (CR10X, Campbell Scientific Inc.) recording 4 hour mean temperatures in 2007/2008.

TABLE 2

Mean monthly N₂-fixation rates ($\mu\text{mol N m}^{-2} \text{hr}^{-1}$) in incubations under field and optimized environmental conditions for each of the principal N₂-fixing cyanobacterial associations in the low arctic tundra landscape near Daring Lake, Northwest Territories, Canada. Acetylene reduction conversion ratios based on optimal conditions are included for each N₂-fixing association. Parentheses indicate standard errors. BSC = biological soil crust.

N ₂ -Fixing Association	Incubation condition	Mean Monthly N ₂ -Fixation rate ($\mu\text{mol N m}^{-2} \text{hr}^{-1}$)			Conversion ratio C ₂ H ₄ /N ₂
		June	July	August	
Hollow BSC	Field	4.28 (0.47)	13.01 (1.30)	11.00 (1.23)	3.49 (0.85)
	Optimal	11.40 (2.26)	25.87 (3.57)	25.05 (4.45)	
Hummock BSC	Field	11.69 (0.90)	13.70 (0.91)	10.70 (0.91)	1.33 (0.40)
	Optimal	28.12 (3.63)	37.08 (4.27)	19.34 (1.43)	
<i>Sphagnum</i> spp.	Field	31.05 (1.87)	33.11 (2.37)	20.69 (1.34)	0.85 (0.12)
	Optimal	n/a	n/a	n/a	
<i>Stereocaulon paschale</i>	Field	56.97 (7.08)	43.45 (8.89)	59.98 (7.24)	1.78 (0.20)
	Optimal	192.10 (23.74)	303.06 (29.20)	217.38 (25.24)	

Modeling N₂-Fixation Potential

Models of N₂-fixation potential were determined for BSCs using N₂-fixation rates and microclimatic data recorded in the *in situ* ambient incubations. The *Sphagnum* spp. N₂-fixation model was based on N₂-fixation rates under controlled laboratory conditions, and the *S. paschale* model was based on N₂-fixation rates recorded during *in situ* ambient incubations and macroclimatic data recorded at a local Daring Lake weather station (~500 m from the research station) (2007–2008; Bob Reid, INAC, unpublished data). Spearman correlations were determined between mean N₂-fixation rate, mean light (PAR), mean incubation temperature, and mean % moisture for each incubation across all *in situ* ambient incubations (2007 and 2008) for Hollow ($n = 54$) and Hummock BSCs ($n = 54$). Temperature had the highest correlation with N₂-fixation for both Hollow ($r = 0.78$) and Hummock BSCs ($r = 0.64$). Light and temperature had a high covariance for Hollow BSCs ($r = 0.84$) and Hummock BSCs ($r = 0.81$); therefore, only temperature and moisture were used in the models. Separate models were determined for high and low moisture conditions. High and low moisture classes were based on percent moisture values above ('high') and below ('low') the median % moisture detected for Hollow or Hummock BSCs incubated in the field over the growing season in 2007 and 2008.

Sphagnum spp. were sampled from the field site in early August 2009, kept cool (~4 °C), and shipped to the lab at the University of Northern British Columbia. The samples were incubated under a range of laboratory conditions reflecting field conditions in 2007–2008 to determine the response of N₂-fixation to temperature and light. Moisture was not included because >70% of *Sphagnum* spp. within the landscape occurred in Wet Sedge Meadows where moisture remains relatively high throughout the growing season and is likely not limiting. *Sphagnum* spp. N₂-fixation rates were significantly correlated with temperature ($r = 0.62$). N₂-fixation rates under light conditions ranging from 0 to 1000 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ were not significantly different (ANOVA, Tukey post hoc, $p = 0.09$); therefore, only temperature was included in the model.

Mean N₂-fixation by *S. paschale* under *in situ* ambient incubations ($n = 27$) was highly correlated with mean % moisture ($r = 0.92$). Days since precipitation was used as the moisture variable for *S. paschale* since direct measurements of field moisture content were not possible. A considerable lag period often occurs following saturation of *Stereocaulon* mats from rainfall events before steady nitrogenase activity is recovered (Crittenden and Kershaw, 1978). Therefore, a 24 hour lag was

incorporated into the days since precipitation variable. The 24 hours following a precipitation event (≥ 1 mm) was coded as 0 and every subsequent day without a precipitation event coded with an increasing value of 1. Days since precipitation was highly correlated with mean % moisture ($r = -0.76$) and with N₂-fixation ($r = -0.84$).

The above models were used to estimate hourly N₂-fixation rates for each association over a growing season based upon microclimate and macroclimatic monitoring in the study landscape. Hourly N₂-fixation rates were summed to provide daily (Fig. 2) and seasonal totals (Table 4). We defined the start of the growing season as the first set of three or more consecutive days with no snow cover and mean air temperature above 0 °C, and the end of the growing season as the first occurrence of three or more consecutive days with mean air and soil surface temperature <0 °C. In 2007 and 2008 these conditions occurred between Julian days 152 and 257 and 144 and 259, respectively. Estimates of N₂-fixation for all of the above growing season days were not possible for every N₂-fixing association due to unavailable microclimatic data. Therefore, the total mean N input for each association was based on the average of 2007 and 2008 estimates over a 103 day growing season from 154 to 257 in both years. Air temperature, snow depth, and precipitation were determined from macroclimatic data from the local Daring Lake weather station (2007–2008; Bob Reid, INAC, unpublished data).

Quantification of N₂-Fixing Associations in the Landscape

The areal extents of each of the N₂-fixing associations within each ecosystem type (Xerophytic Herb Tundra, Heath-Lichen/Heath-Mat Tundra, Birch Hummock, Wet Sedge Meadows) in the study area were determined using line transects in June 2007. Ten parallel transects (~50 m apart, and ~1 km in length) were run from an esker ridge down across a valley and up to an elevated boulder field plateau within the East Daring Lake drainage basin. The variation in topography and therefore of vegetation types within the transect area is typical of the Barrenlands region and representative of the landscape study area. Percent cover of each N₂-fixing association was visually estimated within all 25 × 25 cm² subsections of 1 m² quadrats that were placed every 10 m along each line transect. The dominant ecosystem type in each quadrat was noted, and then the mean percent cover of each of the four principal N₂-fixing associations was visually estimated by two independent observers. The total area of each N₂-fixing association within the landscape was estimated based on its mean % cover

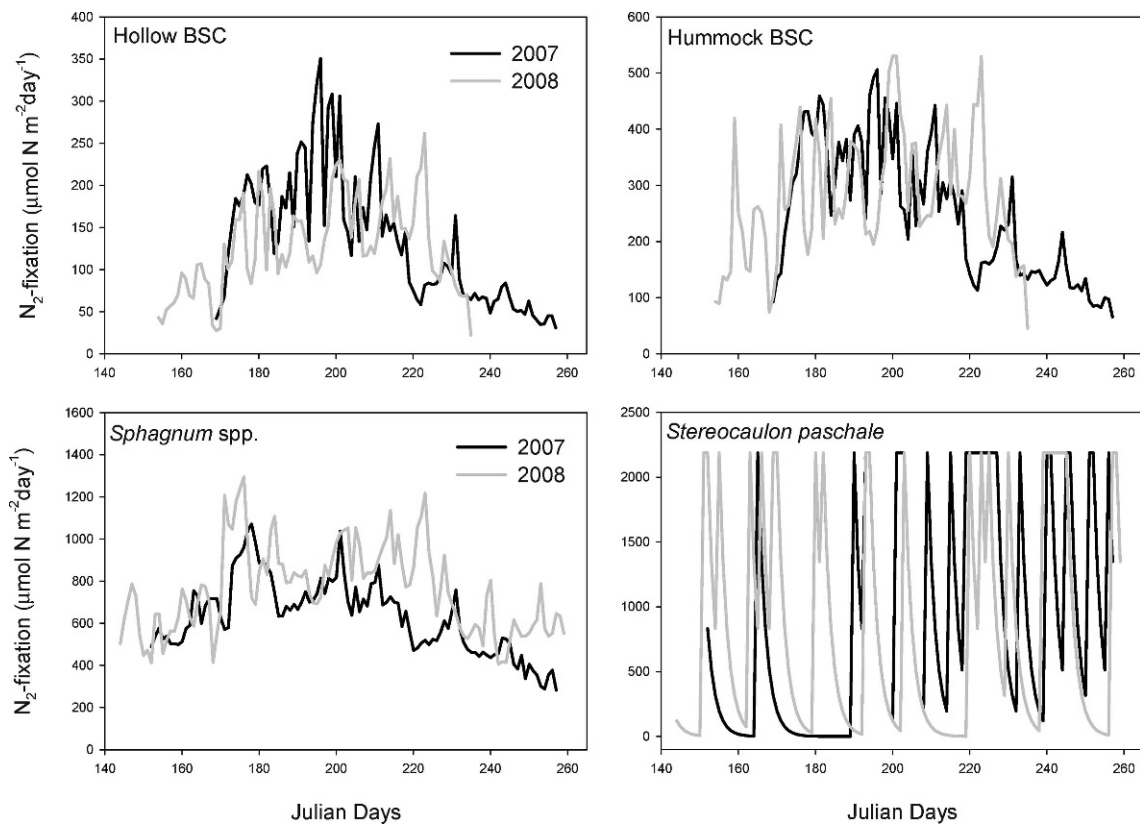


FIGURE 2. Seasonal trends in N_2 -fixation rate ($\mu\text{mol N m}^{-2} \text{day}^{-1}$) estimated from potential N_2 -fixation rate models and field environmental data records for each N_2 -fixing association for 2007 and 2008 at Daring Lake, NWT. See Table 3 for further model details. ARA to N_2 -fixation conversion ratios (Table 2) were applied for each N_2 -fixing association.

within each ecosystem type (determined from transect data) and the total area occupied by each ecosystem type within the 26.7 km² landscape study area (determined from Obst, 2008).

The total mean growing season N input ($\text{kg ha}^{-1} \text{yr}^{-1}$) for each N_2 -fixing association was estimated by averaging the 2007 and 2008 model outputs for Julian days 154–257 in both years. The total N input by each N_2 -fixing association within each ecosystem type was determined by multiplying the mean total growing season N input for each association ($\text{kg ha}^{-1} \text{yr}^{-1}$) by the area (ha) occupied by each association. Total N input for each ecosystem type is the sum of N inputs from all N_2 -fixing associations within a given ecosystem type. Total landscape N input over the growing season was determined by summing N input from all N_2 -fixing associations in each of the ecosystem types over the growing season and dividing by the total landscape study area (26.7 km²).

STATISTICAL ANALYSES

Comparisons of mean N_2 -fixation rates by the principal N_2 -fixing associations over the growing season (June–August) under field and optimal conditions were analyzed using separate factorial analyses of variance (ANOVA) (N_2 -fixing type, month, and their interaction). Logistic regressions were used to develop the models of N_2 -fixation potential based on microclimate. Data from both 2007 and 2008 were used in comparisons of N_2 -fixation rates by the principal N_2 -fixing associations and in the models of N_2 -fixation potential based on microclimate. N_2 -fixation rates were log transformed prior to all statistical analyses (SYSTAT 8.0, Systat Software, Inc.).

Results

*N*₂-FIXATION RATES OF THE PRINCIPAL CYANOBACTERIAL ASSOCIATIONS

Mean monthly N_2 -fixation rates under field conditions differed significantly among N_2 -fixing associations ($F_{(3,2232)} = 156.51$, $P < 0.01$) and were significantly different between months ($F_{(2,2232)} = 3.40$, $P = 0.03$) (Table 2). The interaction of month and N_2 -fixing association was also significant ($F_{(6,2232)} = 27.67$, $P < 0.01$) with patterns of N_2 -fixation over the growing season (June–August) varying among the different associations. The highest rates of N_2 -fixation for all of the associations with the exception of *S. paschale* occurred in July; however, Hollow BSCs had lower rates in June compared with July and August, and *Sphagnum* spp. had lower rates in August compared with June and July.

N_2 -fixation rates under optimal conditions (200 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$, 20 °C) differed among cyanobacterial associations ($F_{(2,154)} = 181.15$, $P < 0.01$) and between months ($F_{(2,154)} = 8.17$, $P < 0.01$), and there was a significant interaction between these two factors ($F_{(4,154)} = 2.70$, $P = 0.03$). The highest N_2 -fixation rates under optimal conditions for all associations were in July (Table 2). The lowest rates under optimal conditions occurred in June for both Hollow BSCs and *S. paschale*, while the lowest rates occurred in August for Hummock BSCs.

Comparison of N_2 -fixation rates under field and optimal conditions clearly indicated that adverse *in situ* environmental factors severely curtailed N_2 -fixation, and that the extent of this constraint varied substantially among cyanobacterial associations.

TABLE 3

Potential N₂-fixation rate logistic regression models based on acetylene reduction (AR) rates in field incubations for each of the principal N₂-fixing associations. Hollow and Hummock BSC data were each separated into two moisture classes as indicated. Environmental variables included in models are surface temperature of Hollow (T_{ho}) and Hummock (T_{hu}) BSCs, *Sphagnum* spp. temperature at 2 cm depth (T_s), and Days since precipitation (D_{sp}). The dependent variable for all models is log acetylene reduction (μmol C₂H₄ m⁻² hr⁻¹).

N ₂ -Fixing Association	Moisture Class	Model	N	F	R ²
Hollow BSC	High (>80%)	$(0.07 \times T_{ho}) + 0.37$	25	80.39	0.77
	Low (<80%)	$(0.05 \times T_{ho}) + 0.69$	25	84.67	0.78
Hummock BSC	High (>35%)	$(0.05 \times T_{hu}) + 0.55$	22	28.05	0.56
	Low (<35%)	$(0.04 \times T_{hu}) + 0.60$	28	28.46	0.50
<i>Sphagnum</i> spp.	None	$(0.04 \times T_s) + 0.99$	14	34.68	0.72
<i>Stereocaulon paschale</i>	None	$(-0.21 \times D_{sp}) + 2.21$	23	49.46	0.69

Note: All models statistically significant, $P < 0.01$.

BSC associations had N₂-fixation rates under optimal conditions that were 2–3 times higher than those observed under field conditions, while rates in *S. paschale* were 4–7 times higher under optimal conditions (Table 2).

LANDSCAPE-SCALE PATTERNS OF N INPUT

Microclimatic Models of Potential N₂-Fixation for Each Cyanobacterial Association

Our simple models of N₂-fixation rates in relation to either temperature and/or moisture explained at least 50% of the variation in the field incubation data (Table 3). N₂-fixation rates were correlated with temperature in the high moisture and low moisture category samples ($R^2 = 0.77$, $P < 0.001$; $R^2 = 0.78$, $P < 0.001$, respectively) of Hollow BSC associations (Table 3). Similarly, N₂-fixation rates were correlated with temperature in the high moisture and low moisture category samples ($R^2 = 0.56$, $P < 0.001$; $R^2 = 0.50$, $P < 0.001$, respectively) of Hummock BSC associations (Table 3). N₂-fixation rates for the *Sphagnum* spp. cyanobacterial associations were also correlated with temperature ($R^2 = 0.72$, $P < 0.001$), while *S. paschale* rates were significantly correlated with days since precipitation ($R^2 = 0.69$, $P < 0.001$) (Table 3).

Seasonal trends of N₂-fixation estimated by using full growing season field microclimatic records in the models indicated that each N₂-fixing association had similar patterns of activity in 2007 and 2008 (Fig. 2). N₂-fixation inputs in Hollow BSC, Hummock BSC, and *Sphagnum* spp. associations fluctuated dynamically during the first half of the season but tended to

generally increase toward peak values in mid to late July, and then to decline fairly steadily afterwards. No clear seasonal trend could be observed for *S. paschale* because estimates were based solely on days since precipitation. The model estimates of mean total N input across the growing season (3 June to 13 September) for each cyanobacterial association ranged from 3.4 kg N ha⁻¹ yr⁻¹ (Hollow BSCs) to 24.9 kg N ha⁻¹ yr⁻¹ (*S. paschale*) for a 103 day growing season (Table 4).

N Input by N₂-Fixing Associations and by Ecosystem Types

No single N₂-fixing association dominated N inputs across all of the ecosystem types. *Stereocaulon paschale* was the largest source of N input in both Xerophytic Herb Tundra and Heath-Lichen/Heath-Mat Tundra followed by Hummock BSC (Table 5). *Sphagnum* spp. was the largest source of N input in both Birch Hummock and Wet Sedge Meadow ecosystems followed by Hollow BSC. Despite having the highest mean N₂-fixation rate, *S. paschale* did not have the highest overall landscape N input (549.84 kg; Fig. 3). *Sphagnum* spp. had the highest N input (1030.72 kg) due to its relatively high mean N₂-fixation rate and greater area within the landscape (50.28 ha) compared with *S. paschale* (22.11 ha) (Fig. 3).

We used the model estimates of growing season N₂-fixation by each cyanobacterial association along with the mapping data of the distribution of ecosystem types in our landscape study area to estimate overall N inputs in each ecosystem type. Total N input per unit area was ~10 times higher in the Wet Sedge Meadow than in any other ecosystem type (Table 5). Our spatially explicit analyses indicate that this effect can be explained by particularly high inputs

TABLE 4

Mean total N fixed over the growing season (3 June to 13 September) based on estimates of N₂-fixation by Hollow BSC, Hummock BSC, *Sphagnum* spp., and *Stereocaulon paschale* at Daring Lake, Northwest Territories, in 2007 and 2008. Microclimatic models were used to predict hourly acetylene reduction rates per m² (See Table 3). ARA to N₂-fixation conversion ratios (Table 2) were applied for each N₂-fixing association. Rates were summed to give total mg N m⁻² yr⁻¹ based on the 2007 and 2008 growing seasons indicated by Julian days. The mean of 2007 and 2008 estimates based on a 103 day growing season (154–257) in both years was used to determine mean total N.

N ₂ -Fixing Association	Year (Julian Days)	Total mg N m ⁻² yr ⁻¹	Mean Total N kg ⁻¹ ha ⁻¹ yr ⁻¹ (Julian Days 154–257)
Hollow BSC	2007 (169–257)	334	3.4
	2008 (154–235)	292	
Hummock BSC	2007 (169–257)	622	7.1
	2008 (154–235)	645	
<i>Sphagnum</i> spp.	2007 (152–257)	1865	20.5
	2008 (144–259)	2460	
<i>Stereocaulon paschale</i>	2007 (152–257)	3150	24.9
	2008 (144–259)	3204	

TABLE 5

The contributions of individual N₂-fixing associations to total biological N input across the selected landscape study area and to N inputs per unit area for each of the major ecosystem types at Daring Lake, Northwest Territories, over the growing season (Julian days 154 to 257).

Ecosystem type	Total area (and proportion) of each ecosystem type within the study landscape (ha)	N ₂ -fixing association	Mean cover of N ₂ -fixing association within each ecosystem type (%)	Area of each N ₂ -fixing association (ha)	N input by each N ₂ -fixing association within each ecosystem type (kg N yr ⁻¹)	Total N input within each ecosystem type (kg N yr ⁻¹)	Total N input per unit area for each ecosystem type (kg N ha ⁻¹ yr ⁻¹)
Xerophytic Herb Tundra	74.58 (5.5%)	Hollow BSC	0.02	0.01	0.03	54.48	0.73
		Hummock BSC	4.44	3.31	23.54		
		<i>Sphagnum</i> spp.	0.28	0.21	4.31		
		<i>Stereocaulon paschale</i>	1.44	1.07	26.61		
Heath-Lichen/Heath-Mat Tundra	568.87 (42.0%)	Hollow BSC	0.36	2.05	7.07	777.19	1.37
		Hummock BSC	4.19	23.84	169.52		
		<i>Sphagnum</i> spp.	0.82	4.66	95.53		
		<i>Stereocaulon paschale</i>	3.57	20.31	505.07	235.39	1.37
Birch Hummock	171.29 (12.6%)	Hollow BSC	4.12	7.06	24.35		
		Hummock BSC	0.53	0.91	6.47		
		<i>Sphagnum</i> spp.	5.33	9.13	187.16		
		<i>Stereocaulon paschale</i>	0.41	0.70	17.41		
Wet Sedge Meadow	68.9 (5.1%)	Hollow BSC	2.39	1.65	5.69	750.24	10.89
		Hummock BSC	0.02	0.01	0.07		
		<i>Sphagnum</i> spp.	52.65	36.28	743.73		
		<i>Stereocaulon paschale</i>	0.04	0.03	0.75		

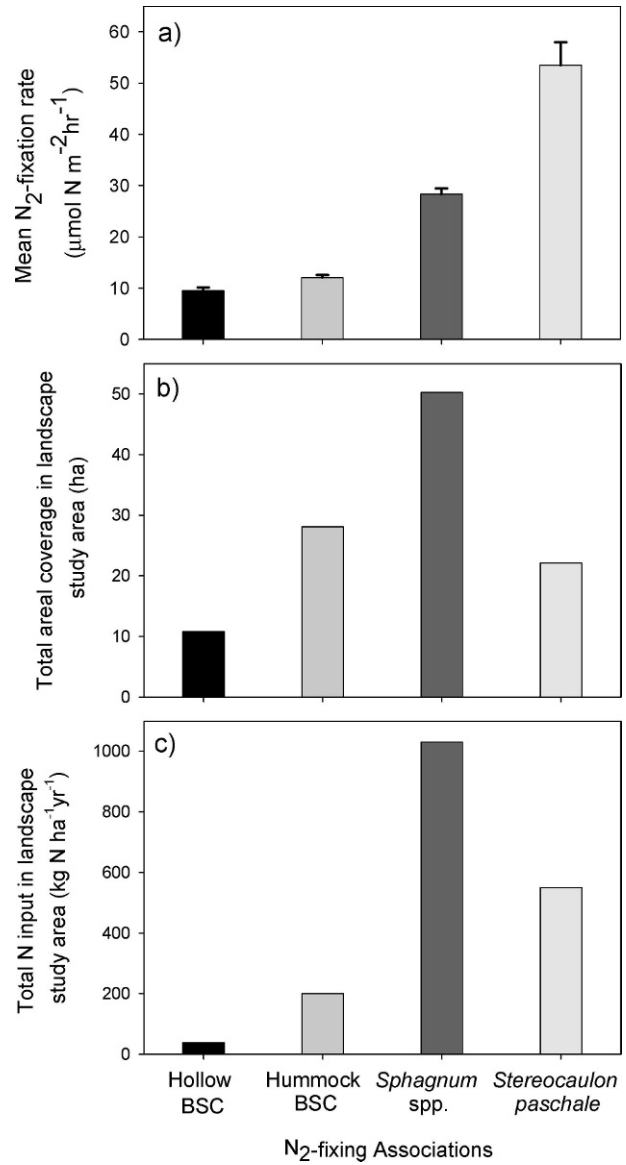


FIGURE 3. (a) Mean N₂-fixation rate measured over the growing seasons of 2007 and 2008 (see Table 2), (b) total area of N₂-fixing associations in landscape study area, and (c) total N input for Hollow biological soil crusts (BSCs), Hummock BSCs, *Sphagnum* spp., and *Stereocaulon paschale* determined by potential N₂-fixation models in the landscape study area at Daring Lake, NWT. Total area of N₂-fixing associations and total N input in landscape study area were calculated from values in Table 5. Error bars in (a) indicate standard error.

by *Sphagnum* spp. cyanobacterial associations due to relatively large fixation rates (Table 4) in combination with relatively high proportional cover of this association in the Wet Sedge Meadow (Table 5). Heath-Lichen/Heath-Mat Tundra had relatively low N inputs per unit area but had the largest total N input in our selected landscape study area because of its abundant coverage in the landscape. Birch Hummock tundra had similar total N₂-fixation rates per unit area to Heath-Lichen/Heath-Mat Tundra, but its coverage was low in the study area, resulting in low total N inputs. Finally, N₂-fixation rates per unit area within Xerophytic Herb Tundra were lowest, and its coverage was also low, resulting in relatively small N inputs into the selected landscape study area. Total N input for 68% the Daring Lake landscape study area over the 103 day growing season was 0.68 kg ha⁻¹ yr⁻¹.



FIGURE 4. Hummock-hollow complexes at Daring Lake, NWT, 17 June 2008. Differences in early season fixation rates between Hollow and Hummock BSC may result from snow covering hollows of hummock-hollow complexes whereas Hummock BSC is exposed.

Discussion

Our study demonstrates that biological N₂-fixation across a low arctic landscape is both temporally and spatially heterogeneous due to the presence of distinct cyanobacterial associations that varied in their responses to seasonal environmental changes, and in their distribution among vegetation types. Our study design integrated individual N₂-fixing association responses to seasonal microclimatic conditions, the abundance of each N₂-fixing association within different ecosystem types, and the prevalence of the ecosystem types within the landscape. By employing this multiscale approach we not only provided a landscape-level estimate of N input via N₂-fixation (0.68 kg ha⁻¹ yr⁻¹), but also identified the ecosystem type (Wet Sedge Meadow), cyanobacterial association (*Sphagnum* spp.), and microclimatic controls (moisture and temperature) that are key to understanding biological N inputs. In addition, we found a significant interaction between growing season month and type of N₂-fixing association, indicating that changes in seasonal progression of N₂-fixation activity vary among cyanobacterial associations. Further, our results highlight the importance of considering both the abundance cover and average N₂-fixation rate of each N₂-fixing association in characterizing the controls on patterns of N input across the landscape, and in estimating the total magnitude of N inputs. For example, the primary importance of *Sphagnum* spp. associations to total landscape N inputs was due to their relatively high rates of N₂-fixation, as well as their high percent cover compared to the other N₂-fixing associations. By contrast, the lichen *S. paschale* was relatively infrequent on the landscape but made the second largest contribution to total N inputs because it had particularly high rates of N₂-fixation (Fig. 3). Together these results provide substantial insights into the principal factors causing both temporal and spatial heterogeneity in biological N₂-fixation inputs in the low Arctic.

MICROCLIMATIC CONTROLS ON SEASONAL AND SPATIAL VARIATION IN N₂-FIXATION

Several studies have detected distinct seasonal patterns in N₂-fixation rates in the Arctic (Alexander and Schell, 1973; Henry and Svoboda, 1986; Chapin et al., 1991; Zielke et al., 2005). Our data (Table 2 and Fig. 2) are consistent with the general pattern of detectable N₂-fixation rates soon after snowmelt (March–June)

followed by the highest rates coinciding with the peak growing season and declining rates in late July to August depending on latitude. Our field measurements of N₂-fixation demonstrate that seasonal patterns vary among the vegetation communities, which may account for the lack of seasonal variation detected in studies that average across both topography and vegetation type (Hobara et al., 2006).

Moisture appears to be one of the most important environmental factors controlling N₂-fixation across various arctic environments (Chapin and Bledsoe, 1992; Nash and Olafsen, 1995; Zielke et al., 2002, 2005). Moisture can affect seasonal patterns of N₂-fixation within individual N₂-fixing communities, and spatial heterogeneity in N₂-fixation is often a reflection of differences in cyanobacterial biomass due to the long-term characteristics of the community moisture regime (Chapin et al., 1991). *Stereocaulon paschale*, which was primarily located on xeric esker tops and well-drained upper esker slopes, had the highest mean rate of N₂-fixation over the growing season, but demonstrated a strong sensitivity to desiccation. Lichens are often established on drier exposed habitats where nitrogenase activity may be reduced to a few comparatively short episodes when moisture conditions are suitable (Crittenden and Kershaw, 1979). Rainfall in July 2008 was 6.7 mm compared to 24.6 mm and 28.1 mm in June and August, respectively. Accordingly, the average percent moisture of *S. paschale* incubated in July was 26% as compared to June (56%) and August (45%), perhaps explaining the relatively low July N₂-fixation rates. The relatively high and consistent N₂-fixation rates associated with *Sphagnum* spp. over the growing season are likely due to the consistently high moisture conditions of the low-lying Wet Sedge Meadow where *Sphagnum* spp. are the dominant vegetation.

Temperature has also been significantly correlated with N₂-fixation rates in the Arctic (Smith, 1984; Lenniham et al., 1994; Liengen and Olsen, 1997b; Zielke et al., 2002). Our strong correlations between N₂-fixation and temperature for Hollow BSC, Hummock BSC, and *Sphagnum* spp. indicate that seasonal temperature fluctuations are important in determining seasonal rates of N₂-fixation. Like Zielke et al. (2005), we also found that temperature was a good predictor of N₂-fixation provided different models were used depending on moisture condition. Hollow BSC had lower field and optimal rates of N₂-fixation in June compared with other cyanobacterial associations (Table 2). Mean Hollow BSC temperature in June was 6.8 °C compared to 9.1 °C for Hummock BSC. Therefore, the lower rates of N₂-fixation for Hollow BSC are likely due to the persistence of snow in these depressions resulting in relatively low temperatures, as well as restricted light inputs that together may impede the recovery and development of cyanobacterial communities in the early growing season (Fig. 4).

Some studies have found N₂-fixation to be light dependent (Granhall and Lid-Torsvik, 1975; Alexander et al., 1978) while others have found little light dependence as photosynthetic rates tend to saturate at relatively low light levels (<500 μmol PAR m⁻² s⁻¹) (Coxson and Kershaw, 1983b; Smith, 1984; Chapin and Bledsoe, 1992; Nash and Olafsen, 1995; Zielke et al., 2002). Varying light conditions (0 to 1000 μmol PAR m⁻² s⁻¹) did not affect *Sphagnum* spp. N₂-fixation rates in our study, supporting the concept that stored energy for N₂-fixation, combined with continuous or near continuous daylight and a limited plant canopy, reduce the potential for light to act as a controlling factor on N₂-fixation in the Arctic (Chapin and Bledsoe, 1992). Nevertheless, remote sensing, repeat photography, and experimental nutrient addition studies all suggest that current warming trends in the low Arctic may be promoting shrub growth and expansion within various topographic positions (Goetz et al., 2005; Sturm et al., 2001;

Chapin et al., 1995). Declining macrolichen abundance in warming sub- and mid-arctic environments may be a function of increased growth and abundance of deciduous shrubs, which may inhibit lichen performance and persistence through shading (Cornelissen et al., 2001). N₂-fixation rates and persistence of other N₂-fixing associations in these environments may also be similarly influenced by reduced light availability.

The higher N₂-fixation rates detected under optimal conditions for all N₂-fixing associations indicate that microclimatic conditions in the field are limiting N₂-fixation for all of the principal N₂-fixing associations. We conclude that climate change scenarios that result in warmer surface temperatures without increased surface desiccation are likely to lead to higher rates of N₂-fixation (Chapin and Bledsoe, 1992).

THE SIGNIFICANCE OF BIOLOGICAL N₂-FIXATION TO N CYCLING IN A LOW ARCTIC LANDSCAPE

Since N availability is commonly a major limitation on tundra plant growth, our results provide important insights to understanding the functioning of low arctic terrestrial ecosystems. Our estimate of mean seasonal N₂-fixation in Birch Hummock (Table 5; 1.4 kg N ha⁻¹ yr⁻¹ over 103 days) is ~1/300th the late summer rate of gross N mineralization by soil microbes in the same ecosystem type (Buckeridge et al., 2010). Therefore, internal recycling of N from soil organic matter is undoubtedly the critical N supply process within the Birch Hummock ecosystem type at least. Nevertheless our data suggest a significant influence of N₂-fixation on N cycling and carbon uptake at a larger scale. N₂-fixation during the growing season was highest in the Wet Sedge Meadow, which is also the ecosystem with the largest annual plant primary production in this landscape (Nobrega and Grogan, 2008). Nutrient inputs associated with run-off and leachates from higher elevation ecosystems toward the valley floor where wet sedge ecosystems predominate may facilitate the high rates of primary production there. Our data here suggest that in addition to that process, *in situ* N₂-fixation inputs may be an important pathway supplying N to support the high primary productivity of this ecosystem type.

Total biological N₂-fixation input across the study landscape area was estimated at 0.68 kg N ha⁻¹ yr⁻¹. Previous estimates of arctic N₂-fixation inputs range from 0.06 to 3 kg N ha⁻¹ yr⁻¹, with the majority of estimates ranging from 0.10 to 1.20 kg N ha⁻¹ yr⁻¹ (Alexander and Schell, 1973; Barsdate and Alexander, 1975; Chapin and Bledsoe, 1992; Hobarra et al., 2006). Summertime mean atmospheric N inputs from wet deposition at the nearest monitoring station (63.52°N, 116.00°W) to Daring Lake (~240 km away) were 0.39 kg N ha⁻¹ yr⁻¹ (1991–2006; CAPMon, Environment Canada, unpublished data). Wintertime atmospheric N deposition inputs as total inorganic N accumulation in ambient snow packs (0.3 m) at Daring Lake in 2007 were 0.05 kg N ha⁻¹ (Buckeridge and Grogan, 2010). Together, these numbers suggest that total biological N₂-fixation input for the landscape study area at Daring Lake is approximately twice the amount of N deposited via atmospheric deposition. While some studies have found N₂-fixation contributed 80% or higher to total landscape N inputs (Hobarra et al., 2006; Solheim et al., 2006), other studies, including ours, have found the contribution of N₂-fixation to ecosystem N inputs is approximately 50–70% (Chapin and Bledsoe, 1992; Henry and Svoboda, 1986).

We found N₂-fixation across a low arctic tundra landscape was concentrated in the Wet Sedge Meadow ecosystem type where N₂-fixation per unit area was ~10 times higher than in any of the

other ecosystem types (Table 5). Of the four principal N₂-fixing associations, *Sphagnum* spp., which had the highest percent cover in Wet Sedge Meadows, made the largest contribution (55.2%) to total N input. Several other studies have also found the highest rates of N₂-fixation in arctic landscapes are associated with cyanobacteria moss associations (Alexander and Schell, 1973; Henry and Svoboda, 1986; Solheim et al., 1996).

Five methodological constraints may have affected our landscape estimates of biological N₂-fixation inputs. Firstly, conversion ratios can vary depending on the operating environment of a given N₂-fixing association (Millbank, 1981; Gunther, 1989), and seasonal variation in conversion ratios has been detected for free-living cyanobacteria from high arctic habitats (Liengen, 1999b). Secondly, we used visual estimates of abundance for the N₂-fixing associations without accounting for variations in cyanobacterial biomass that can impact rates of N₂-fixation. Thirdly, the ecosystem types in our analysis account for only 68% of the Daring Lake study area. Some excluded ecosystem types (Exposed Sand and Gravel, and Rocky Outcrops) probably contribute little or nothing to landscape N input. However, other ecosystem types such as Dry Sedge Meadows (8.2%) may contain considerable *Sphagnum* spp. cyanobacterial associations and therefore may make significant contributions to landscape N input, albeit for a limited duration due to less favorable microclimatic conditions. Fourthly, our estimates of modeled N inputs would have been improved by more accurate quantification of spatial variability in soil surface microclimate by using a much larger number of climate sensors. This is a limitation that is common to many studies of arctic and subarctic ecosystems (Rouse, 1976; Young et al., 1997). Fortunately, the type of conditions that favor N₂-fixation by most cyanobacterial associations (i.e. during or immediately following growing season precipitation events) will tend to minimize between site variability in soil surface microclimate, reducing the impact of this factor on our estimates. Fifthly, we used a 103 day growing season as the basis for yearly N input. However, N₂-fixation likely occurs outside of this period whenever microclimatic conditions are favorable (Davey, 1983; Liengen, 1999a; Zielke et al., 2002; Hobarra et al., 2006). In summary we conclude that, provided our conversion ratios, percent cover of cyanobacterial associations, and models of potential N₂-fixation are sufficiently accurate, our estimates of ecosystem N₂-fixation inputs and of total landscape-level N input over the growing season are minimum values.

Acknowledgments

We thank Kelvin Kotchilea, Rebecca Carmichael, Ryan Mercredi, Tyanna Steinwand, and Mat Vankoughnett for assistance in the field, and Steve Matthews and Andy Kritsch for logistical support. We also thank Burkhard Büdel, Olivia Lee, and Curtis Bjork for identification of cyanobacteria, bryophyte, and soil crust species. Elyn Humphreys' contribution of microclimate data from the Wet Sedge Meadow and John McKay's provision of the atmospheric N deposition data are greatly appreciated. This study was supported by NSERC and the Canadian Federal Government as part of the International Polar Year 2007–2008 in collaboration with Climate Change Impacts on Canadian Arctic Tundra (CiCAT) project.

References Cited

Alexander, V., 1974: A synthesis of the IBP tundra biome circumpolar study of nitrogen fixation. *In* Holding, A. J., Heal, O. W., MacLean, S. F., and Flanagan, P. W. (eds.), *Soil*

- Organisms and Decomposition in Tundra*. Stockholm, Sweden: Tundra Biome Steering Committee, 109–121.
- Alexander, V., and Schell, D. M., 1973: Seasonal and spatial variation of nitrogen fixation in the Barrow, Alaska, tundra. *Arctic and Alpine Research*, 5: 77–88.
- 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*. New York: Springer-Verlag, 539–558.
- 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., and Granhall, U., 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.
- Buckeridge, K. M., and Grogan, P., 2010: Deepened snow increases late thaw biogeochemical pulses in mesic low arctic tundra. *Biogeochemistry*, 101: 105–121.
- Buckeridge, K. M., Zufelt, E., Chu, H., and Grogan, P., 2010: Soil nitrogen cycling rates in low arctic shrub tundra are enhanced by litter feedbacks. *Plant Soil*, 330: 407–421.
- Chapin, D. M., and Bledsoe, C., 1992: Nitrogen fixation in arctic plant communities. In Chapin, R. 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., Knute, J., Nadelhoffer, J., and Laundre, J. A., 1995: Responses of arctic tundra to experimental and observed changes in climate. *Ecology*, 76: 694–711.
- Cornelissen, J. H. C., Callaghan, T. V., Alatalo, J. M., Michelsen, A., Graglia, E., Hartley, A. E., Hik, D. S., Hobbie, S. E., Press, M. C., Robinson, C. H., Henry, G. H. R., Shaver, G. R., Phoenix, G. K., Gwynn Jones, D., Jonasson, S., Chapin, F. S., III, Molau, U., Neil, C., Lee, J. A., Mellillo, J. M., Sveinbjornsson, B., and Aerts, R., 2001: Global change and arctic ecosystems: Is lichen decline a function of increases in vascular plant biomass? *Journal of Ecology*, 89: 984–994.
- Coxson, D. S., 1991: Impedance measurement of thallus moisture content in lichens. *Lichenologist*, 23: 77–84.
- Coxson, D. S., and Kershaw, K. A., 1983a: Rehydration response of nitrogenase activity in terrestrial *Nostoc commune* from Stipa-Bouteloa grassland. *Canadian Journal of Botany*, 61: 2658–2668.
- Coxson, D. S., and Kershaw, K. A., 1983b: The pattern of *in situ* summer nitrogenase activity in terrestrial *Nostoc commune* from Stipa-Bouteloa grassland, southern Alberta. *Canadian Journal of Botany*, 61: 2686–2693.
- Crittenden, P. D., and Kershaw, K. A., 1978: Discovering the role of lichens in the nitrogen cycle in the boreal-arctic ecosystem. *The Bryologist*, 81: 258–267.
- Crittenden, P. D., and Kershaw, K. A., 1979: Studies on lichen-dominated systems. XXII. The environmental control of nitrogenase activity in *Stereocaulon paschale* in spruce-lichen woodland. *Canadian Journal of Botany*, 57: 236–254.
- Davey, A., 1983: Effects of abiotic factors on nitrogen fixation by blue-green algae in Antarctica. *Polar Biology*, 2: 95–100.
- Dickson, L. G., 2000: Constraints to nitrogen fixation by cryptogamic crusts in a polar desert ecosystem, Devon Island, N.W.T., Canada. *Arctic and Alpine Research*, 32: 40–45.
- Goetz, S. J., Bunn, A. J., Fiske, G. J., and Houghton, R. A., 2005: Satellite observed photosynthetic trends across boreal North America associated with climate and fire disturbance. *Proceedings of the National Academy of Sciences*, 102: 13521–13525.
- Granhall, U., and Lid-Torsvik, V., 1975: Nitrogen fixation by bacteria and free-living blue-green algae in tundra areas. In Wielgolaskie, F. E. (ed.), *Fennoscandian Tundra Ecosystems, Part 1, vol 16*. New York: Springer-Verlag, 306–315.
- Granhall, U., and Selander, H., 1973: Nitrogen fixation in a subarctic mire. *Oikos*, 24: 8–15.
- Gunther, A. J., 1989: Nitrogen fixation by lichens in a subarctic Alaskan watershed. *The Bryologist*, 92: 202–208.
- Henry, G. H. R., and Svoboda, J., 1986: Dinitrogen fixation (acetylene reduction) in high arctic sedge meadow communities. *Arctic and Alpine Research*, 18: 181–187.
- Hobara, S., McCalley, C., Koba, K., Giblin, A. E., Weiss, M. S., Gettel, G. M., and Shaver, G. R., 2006: Nitrogen fixation in surface soils and vegetation in an Arctic tundra watershed: a key source of atmospheric nitrogen. *Arctic, Antarctic, and Alpine Research*, 38: 363–372.
- INAC, 2007, *Daily and hourly weather data from the weather station at Daring Lake (raw data on Excel spread sheets)*. Yellowknife, NT: Indian and Northern Affairs Canada (INAC), Water Resources Division (courtesy of B. Reid).
- Lafleur, P. M., and Humphreys, E. R., 2008: Spring warming and carbon dioxide exchange over low arctic tundra in central Canada. *Global Change Biology*, 14: 1–17.
- Lenihan, R., Chapin, D. M., and Dickson, L. G., 1994: Nitrogen fixation and photosynthesis in high arctic forms of *Nostoc commune*. *Canadian Journal of Botany*, 72: 940–945.
- Liengen, T., 1999a: Environmental factors influencing the nitrogen fixation activity of free-living terrestrial cyanobacteria from a high arctic area, Spitsbergen. *Canadian Journal of Microbiology*, 45: 573–581.
- Liengen, T., 1999b: Conversion factor between acetylene reduction and nitrogen fixation in free-living cyanobacteria from high arctic habitats. *Canadian Journal of Microbiology*, 45: 223–229.
- Liengen, T., and Olsen, R. A., 1997a: Seasonal and site-specific variations in nitrogen fixation in a high Arctic area, Ny-Alesund, Spitsbergen. *Canadian Journal of Microbiology*, 43: 759–769.
- Liengen, T., and Olsen, R. A., 1997b: Nitrogen fixation by free-living cyanobacteria from different coastal sites in a high Arctic tundra, Spitsbergen. *Arctic and Alpine Research*, 29: 470–477.
- Millbank, J. W., 1981: The assessment of nitrogen fixation and throughput by lichens I. The use of a controlled environment chamber to relate acetylene reduction estimates to nitrogen fixation. *New Phytologist*, 89: 647–655.
- Nadelhoffer, K. J., Giblin, A. E., Shaver, G. R., and Linkins, A. E., 1992: Microbial processes and plant nutrient availability in arctic soils. In Chapin, R. 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, Inc., 281–300.
- Nash, T. H., III, and Olafsen, A. G., 1995: Climate change and the ecophysiological response of arctic lichens. *Lichenologist*, 27: 559–565.
- Nobrega, S., and Grogan, P., 2008: Landscape and ecosystem-level controls on net carbon dioxide exchange along a natural moisture gradient in Canadian low arctic tundra. *Ecosystems*, 11: 377–396.
- Obst, J., 2008: Classification of land cover, vegetation communities, ecosystems and habitats in East Daring Lake Basin, Northwest Territories. Prepared for Department of Environment and Natural Resources, Wildlife Division Government of the Northwest Territories and Environment and Conservation Division, Indian and Northern Affairs Canada, Yellowknife, NT. Contact: Steve_Matthews@gov.nt.ca.
- Rai, A. N., Soderback, E., and Bergman, B., 2000: Cyanobacterium-plant symbioses. *New Phytologist*, 147: 449–481.
- Rouse, W. R., 1976: Microclimatic changes accompanying burning in subarctic lichen woodland. *Arctic and Alpine Research*, 8: 357–376.

- Schell, D. M., and Alexander, V., 1973: Nitrogen fixation in arctic coastal tundra in relation to vegetation and micro-relief. *Arctic*, 26: 130–137.
- Shaver, G. R., and Chapin, F. S., III, 1980: Responses to fertilization by various plant growth forms in an Alaskan tundra: nutrient accumulation and growth. *Ecology*, 61: 662–675.
- Smith, V. R., 1984: Effects of abiotic factors on acetylene reduction by cyanobacteria epiphytic on moss at a subarctic island. *Applied and Environmental Microbiology*, 48: 594–600.
- Solheim, B., Endal, A., and Vigstad, H., 1996: Nitrogen fixation in arctic vegetation and soils from Svalbard, Norway. *Polar Biology*, 16: 35–40.
- Solheim, B., Zielke, M., Bjerke, J. W., and Rozema, J., 2006: Effects of enhanced UV-B radiation on nitrogen fixation in arctic ecosystems. *Plant Ecology*, 182: 109–118.
- Stewart, W. D. P., Fritzgerald, G. P., and Burris, R. H., 1967: *In situ* studies on N₂ fixation using the acetylene reduction technique. *Proceedings of the National Academy of Sciences of the USA*, 58: 2071–2078.
- Sturm, M., Racine, C., and Tape, K., 2001: Climate change—Increasing shrub abundance in the Arctic. *Nature*, 411: 546–547.
- Turetsky, M. R., 2003: The role of bryophytes in carbon and nitrogen cycling. *The Bryologist*, 106: 395–409.
- Walker, D. A., 2000: Hierarchical subdivision of arctic tundra based on vegetation response to climate, parent material, and topography. *Global Change Biology*, 6: 19–34.
- Young, K. L., Woo, M., and Edlund, S. A., 1997: Influence of local topography, soils, and vegetation on microclimate and hydrology at a high arctic site, Ellesmere Island, Canada. *Arctic and Alpine Research*, 29: 270–284.
- 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.
- Zielke, M., Solheim, B., Spjelkavik, S., and Olsen, R. A., 2005: Nitrogen fixation in the High Arctic: role of vegetation and environmental conditions. *Arctic, Antarctic, and Alpine Research*, 37: 372–378.

MS accepted October 2010