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Published in:
Ecosphere (Washington, D.C.)

DOI:
10.1002/ecs2.2117

Publication date:
2018

Document version
Publisher's PDF, also known as Version of record

esa

ECOSPHERE

What drives biological nitrogen fixation in high arctic tundra: Moisture or temperature?

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Citation: Rousk, K., P. L. Sorensen, and A. Michelsen. 2018. What drives biological nitrogen fixation in high arctic tundra: Moisture or temperature? Ecosphere 9(2):e02117. 10.1002/ecs2.2117

Abstract. Biological nitrogen (N_2) fixation is one of the main sources of available N for pristine ecosystems such as subarctic and arctic tundra. Although this has been acknowledged more than a decade ago, few attempts have been undertaken to identify the foremost driver of N_2 fixation in the high Arctic. Here, we report results from in situ measurements of N_2 fixation throughout the main growing period (June–August) in high arctic tundra, Greenland, in climate change treatments, shading and warming, and control. Nitrogen fixation was also measured in cores that received additional water prior to the measurements. The climate change field treatments did not lead to significant changes in any measured parameters; however, N_2 fixation was promoted by adding water, and moisture was the most important factor influencing N_2 fixation in all climate change field treatments. Maximum N_2 fixation rates were measured below 14°C soil temperature, which is much lower than the theoretical and previously reported temperature optimum for the nitrogenase enzyme. Diazotroph (N_2 fixing bacteria) communities are adapted to low temperatures in high arctic settings, and increased temperature in a future climate may lead to decreased N_2 fixation rates, or to a shift in diazotroph communities.

Key words: arctic; bryophytes; climate change; cyanobacteria; heath tundra; nitrogen fixation; nitrogenase.

Received 3 October 2017; revised 20 January 2018; accepted 24 January 2018. Corresponding Editor: Yude Pan.

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INTRODUCTION

Nitrogen is usually the primary limiting nutrient for plant growth in subarctic and arctic tundra (Shaver and Chapin 1980, Michelsen et al. 2012). While atmospheric N deposition is one major source of plant available N in many ecosystems, N deposition in pristine ecosystems, such as subarctic and arctic tundra, is low (<2 kg $N \cdot ha^{-1} \cdot yr^{-1}$; Van Cleve and Alexander 1981, Peñuelas et al. 2013) and is likely not sufficient to cover plant-N demand. Here, fixation of atmospheric N_2 is a large source of plant available N and is performed by free-living N_2 fixing bacteria (diazotrophs), and diazotrophs associated with lichens and mosses (Hobara et al. 2006, Rousk et al. 2016). In boreal forests, subarctic and arctic tundra, moss-associated N_2 fixation can exceed N deposition rates, mostly due to the high coverage and biomass of mosses (Van Cleve et al. 1983, Turetsky 2003, Gavazov et al. 2010, Michelsen et al. 2012). However, N_2 fixation is limited by the availability of molybdenum and phosphorus (Rousk et al. 2017*a*), as well as by extreme temperatures (Gundale et al. 2012, Stewart et al. 2014) and low moisture conditions (Rousk et al. 2014, Stewart et al. 2014). While it has been shown in laboratory (Rousk et al. 2014, 2017*b*), field (Rousk et al. 2015), and modeling (Rousk and Michelsen 2017) approaches that moisture is the major factor driving moss-associated N_2 fixation, those studies have been conducted almost exclusively in boreal forests and subarctic tundra, and attempts to extend this observation to

arctic ecosystems have been limited. Studies that have been conducted in the high Arctic on free-living- as well as on moss and lichen-associated diazotrophs have shown a strong moisture dependence of N_2 fixation (Zielke et al. 2005, Stewart et al. 2011, 2014). On the other hand, Hobara et al. (2006) found an increase in N_2 fixation in plant-soil cores in arctic tundra with temperature up to 30°C, while a positive effect of moisture was not found. Thus, the primary driver (temperature or moisture) of N_2 fixation in the high Arctic remains unspecified.

Arctic ecosystems are among the most sensitive systems to changes in temperature (Seddon et al. 2016, see also Schuur et al. 2015). Increased temperatures resulting in drying-out of mosses in a future climate will lead to limited moisture availability for cyanobacteria within the moss carpet. This will likely lead to reduced N_2 fixation rates (Rousk et al. 2014) and, with that, reduced N input via the N_2 fixation pathway. On the other hand, as a component of warming, summer rainfall is likely to double in the high Arctic by the end of this century (Bintanja and Andry 2017), which could counteract surface drying. In addition to direct temperature effects on N_2 fixation, a warmer climate will also lead to increased thaw depth of permafrost in high arctic settings (Schuur et al. 2015), freeing up N pools (e.g., Keuper et al. 2012) that could inhibit N_2 fixation (e.g., Rousk et al. 2013).

A changing climate will enhance air and soil temperatures on the one hand, but it will also lead to increased shading of lichens, mosses, and free-living diazotrophs from faster growing vegetation and expanding shrubs (Hollister et al. 2005, Wahren et al. 2005). Light is another notable driver of N_2 fixation and can limit diazotroph activity in the tropics (Taylor et al. 2017) as well as in the subarctic (e.g., Sorensen et al. 2012). Given that few diazotroph-associated shrubs grow in the high Arctic, and these are unlikely to expand their range considerably also in the nearer future, the loss of the main N_2 fixing associations as a result of shading and exclusive competition by shrubs (Hartley et al. 2012) will decrease N_2 fixation potential in these systems. Yet, the effects of shading on N_2 fixation in the Arctic have hardly been addressed. Thus, to assess the effects of climate change factors (warming, shading) on N_2 fixation in the Arctic, we utilized a field experiment in a high arctic heath in which climate change was simulated by increasing summer air and soil temperatures using open top chambers as well as by a shading treatment, reducing light input and temperature. We hypothesized that (1) N_2 fixation is highest in the warming treatment if moisture is not limiting, and (2) N_2 fixation is lowest in the shading treatment. To address these hypotheses, we measured N_2 fixation in situ in the climate change field treatments at different times in the growing season. We further divided plots into treatments with added water and without added water before the N_2 fixation measurements to assess how increased precipitation, in combination with shading and warming, in a future climate will affect N_2 fixation. To further test whether the climate change treatments lead to changes in soil N availability via changes in N_2 fixation, we also assessed the soil N pools.

Materials and Methods

Field sites and experimental set-up

The field experiment was established in a mesic-dry heath dominated by *Salix arctica* on Zackenberg, Greenland, in 2004 and has been maintained since. The experimental site is located in the center of the valley of Zackenberg in North-Eastern Greenland, near the Zackenberg Research Station (74°30′ N, 21°00′ W). The climate is continental high Arctic. Average air temperature is −20°, 7.0°, and −9.0°C in January, July, and whole year, respectively, and total annual precipitation is 211 mm but ranging from 93 to 310 mm (data 1997–2014, Jensen et al. 2016), with the most precipitation falling as snow (Fig. 1, see also Appendix S1: Fig. S1). In June, July, and August 2010, the period in which we conducted our measurements, air temperature was 1.9°, 5.3°, and 5.3°C (long-term average 2003–2014: 1.5°, 6.8°, and 5.6°C), and precipitation was 13, 1, and 2 mm (long-term average 2003–2014: 5, 13, and 20 mm). The bedrock is characterized by non-calcareous sandy fluvial sediments, and the soil is classified as a Typic Psammoturbels (Turbic Cryosol) with a pH between 5 and 7. The permafrost is continuous, and the active layer is <1 m deep (60–70 cm during the measuring campaigns, Jensen and Rasch 2011, Elberling et al. 2013). The plots are rather exposed (Appendix S1: Fig. S2A), with a maximum snow cover of 80–95 cm. Besides the

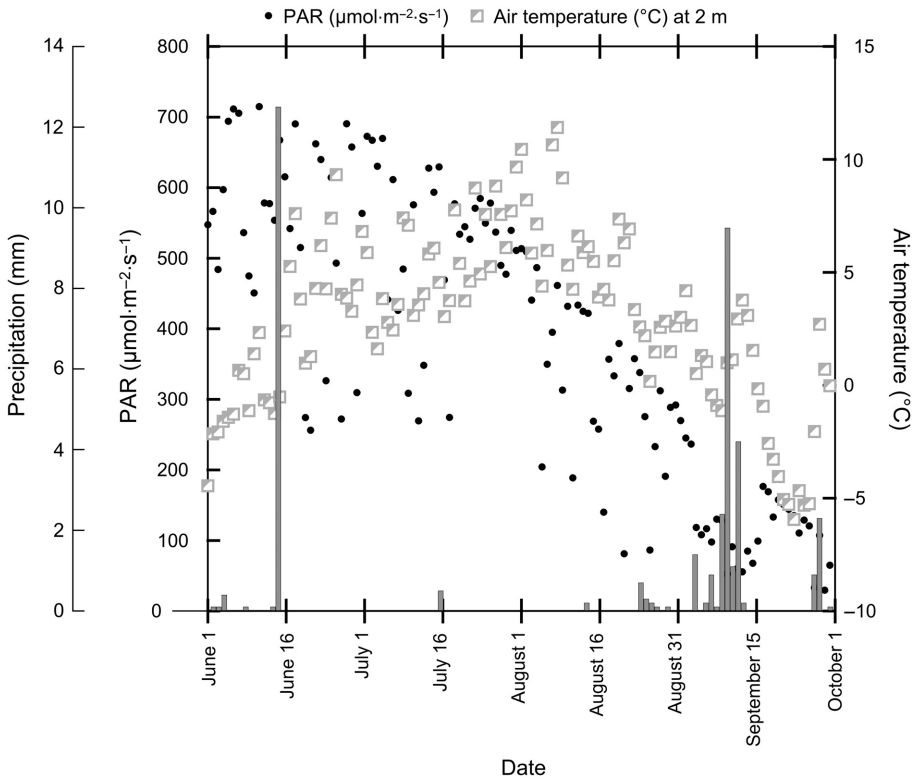

Fig. 1. Daily mean air temperature (°C, gray symbols) at 2 m height, precipitation (accumulated mm/day, gray bars), and daily mean photosynthetic active radiation (PAR, μmol·m^{-2}·s^{-1}, black circles) 1 June to 30 September 2010 at Zackenberg Research Station, NE Greenland.

prostrate *Salix arctica* Pall., other low woody plants are *Vaccinium uliginosum* L., *Cassiope tetragona* D. Don (L.), and *Dryas* spp. and a few herbaceous species such as *Luzula arctica* Blytt, *Polygonum viviparum* L., and *Arctagrostis latifolia* (R. Br.) Griseb. (Campioli et al. 2013).

The field experimental plots consist of five replicate blocks each with the treatments (1) control, (2) shading with sackcloth, and (3) warming by open top chambers. Warming was achieved by small (1.2 × 1.2 m, 50 cm high) dome-shaped open top chambers of 0.05-mm polyethylene film, constructed with polyvinyl chloride (PVC) tubes, with an opening of ~50 cm diameter at the top. The chambers were in place each year from late June to the end of August. Late summer canopy temperature is enhanced by 1.3°C and soil temperature at 2 cm depth by 0.3°C (Campioli et al. 2013), a moderate increase simulating surface temperature projections for high arctic systems for the mid-21st century (IPCC 2013). Shading with sackcloth in chambers of similar constructions with PVC tubes reduces light with 50% and air and soil temperature by 1.5–2.0°C (Fig. 2, see also Ellebjerg et al. 2008). The reduction in incoming light is similar to the reduction caused by taller shrubs such as *Betula nana* and simulates the expansion of canopy-forming shrubs into high-latitude ecosystems.

Vegetation cover

The vegetation cover in each plot was estimated with the pin-point intercept method 23 July 2010 (Jonasson 1983). For this, a pin was passed vertically at 100 points (2 cm spaced) in a 0.5 × 0.5 m frame and every vascular plant species, mosses, lichens, and litter touched by the pin was recorded.

Dependence of N_2 fixation on moisture

Nitrogen fixation was measured using the acetylene reduction assay (ARA) in situ in 2010

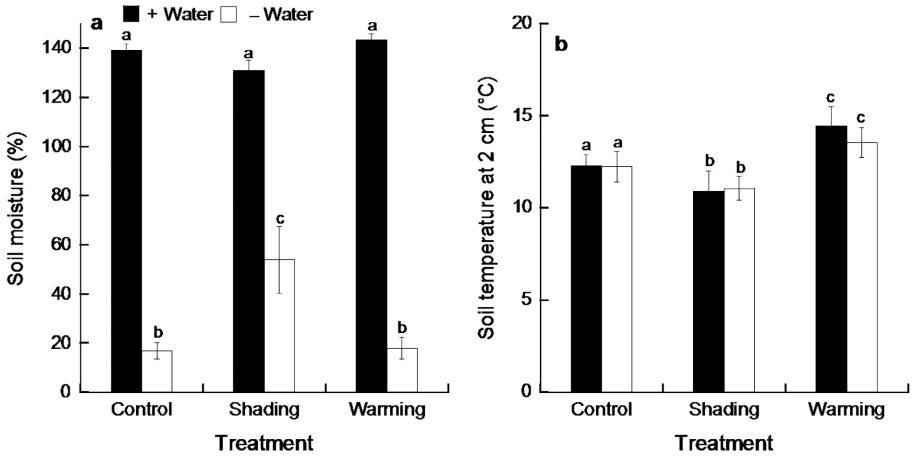

Fig. 2. (a) Soil moisture (%) and (b) soil temperature at 2 cm depth (°C) in the climate change field treatments control, shading, and warming in high arctic tundra, NE Greenland, averaged across the five measuring campaigns in 2010. Given are means ± SE (n = 5) for the plots with water added (black bars) and the plots where no water was added (white bars). Different lowercase letters indicate significant differences between the water treatments and the climate change field treatments.

in the climate change field experiment described above, in which diazotrophs associated with mosses, and free-living diazotrophs are the dominant N_2 fixers (Fig. 3; Appendix S1: Fig. S2). The ARA is a measure of the nitrogenase enzyme

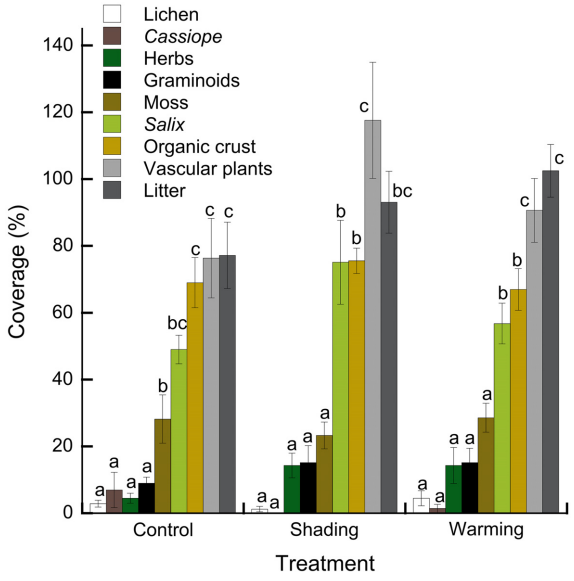

Fig. 3. Coverage of vegetation and litter in the climate change field treatments control, shading, and warming in high arctic tundra, NE Greenland (mean ± SE, n = 5). Different lowercase letters indicate significant differences between plant species.

reducing N_2 to ammonia, as well as reducing acetylene to ethylene. To assess the effects of increased precipitation on N_2 fixation in the field, we established two cores with a diameter of 5 cm and a depth of 7 cm in each experimental field plot (n = 5, Appendix S1: Fig. S2B). The top of the cores were dominated by free-living diazotrophs (organic crust), including lichens and mosses (Appendix S1: Fig. S2B). We placed the cores in nylon mesh bags (50 mm mesh size; Sintab, Oxie, Sweden) and set them carefully back into the hole in the ground, from where the core originated, whenever we were not doing assays and ensured close contact between the core and the surrounding substrate. One core in each plot was with no further treatment, and one core was watered with 10 mL deionized water the day before each ARA measurement, and again immediately before the ARA incubations. This is similar to incidents of 5 + 5 mm precipitation and hence comparable to input in observed precipitation events in mid-June and early September, and the cumulative addition of 50 mm makes up slightly more than a doubling of average current June–September precipitation of 38 mm. For the ARA, samples were placed in closed, transparent PVC containers with a volume of 0.5 L (Appendix S1: Fig. S2). A 20-mL vial containing calcium carbide was placed in each container, and water was added with a syringe through a rubber septum to induce the development of 10% (vol.)

acetylene in the container. The containers were kept in the field plots during the ARAs. Ten milliliters of gas samples was taken 1 min and 2 h after incubation with acetylene and analyzed for acetylene and ethylene on a gas chromatograph (Shimadzu GC-14B, Tokyo, Japan). Acetylene reduction was measured five times during the growing season from mid-July to August 2010 in control, shaded, and warmed plots. Soil temperature at 2 cm depth was measured just before the ARA incubations. Soil moisture was determined gravimetrically. The temperature in the containers during the ARA incubations was not different between the three field treatments and between the water addition treatments (no water addition: 18.4 ± 1.9, 15.6 ± 1.8, 17.9 ± 1.6°C for the control, shading, and warming treatment, respectively; water addition: 18.6 ± 1.7, 15.5 ± 1.6, 17.9 ± 1.6°C for the control, shading, and warming treatment, respectively). After the last measuring campaign in August, the top 2 cm (including organic crust) and the lower part (2–7 cm depth, including roots) of the cores were dried and analyzed for total N and carbon (C) content on an Isoprime isotope ratio mass spectrometer (Elementar UK Ltd., Cheadle Hulme, Stockport, UK) coupled to an Eurovector CN analyzer.

Soil sampling and analyses

Soil was sampled (two cores per plot) with a 4 cm diameter auger to a depth of 5 cm. The soil was homogenized and subjected to chloroform fumigation–extraction and analyzed as in Sorensen et al. (2008). In brief, 10 g of the sorted, fresh soil was fumigated with chloroform for 24 h to release the N and C in the soil microbial biomass, after which the soil was extracted for 1 h in 50 mL demineralized H_2O. The extracts were filtered through Whatman GF-D filters and frozen until analysis. Another 10 g fresh soil was extracted with 50 mL demineralized H_2O to recover soil inorganic N with a Fiastar 5000 Flow Analyzer and dissolved organic carbon (DOC) with a Shimadzu Total Organic Carbon Analyzer. To obtain microbial N, 5 mL of fumigated and unfumigated, demineralized H_2O-extracts was digested in persulfate and analyzed with a Fiastar 5000 Flow Analyzer. Microbial N and C contents were calculated by subtracting the N and C in digested, unfumigated extracts from that in the digested, fumigated extracts. The microbial N and C contents were calculated by assuming an extractability of 0.4 (for N) and 0.45 (for C). Ten grams of fresh soil was dried at 70°C to constant weight to determine the soil moisture content.

Statistical analyses

To test for significant differences in vegetation ground cover, soil temperature, soil moisture, and other soil and soil microbial measures between the climate change field treatments, we ran one-way ANOVAs followed by Tukey's post hoc test. Differences in ARA between measuring campaigns and the climate change field treatments were tested with repeated-measures ANOVA, with water and field treatment as factors, followed by Tukey's post hoc test. ANCOVAs were run to test for the effects of the added water on the relationship between ARA and soil moisture and soil temperature. Finally, to assess the importance of soil moisture, soil temperature, and the water addition treatment on ARA, we performed backwards stepwise multiple regression analyses. All statistical analyses were performed in R 3.0.3 (R Developmental Core Team 2014).

Results

Vegetation ground cover

No differences in ground cover of different functional plant groups between the climate change field treatments were found. However, vascular plants taken together and litter made up most of the ground cover in all treatments ($F_{7,32} > 16$ for all treatments, $P < 0.0001$, Fig. 3).

Nitrogen fixation during the growing season and the effects of water addition

Acetylene reduction was higher in the first two measurements in July (17 July, 21 July) than later in the season (30 July, 3 August). The highest activity was found at the first measuring campaign ($F_{4,120} = 13.55$, $P < 0.0001$, Fig. 4). The plots receiving water before the ARA measurements had higher N_2 fixation rates than the plots without any water added in the control and warming treatments throughout the measuring campaigns and in the shading treatment only at the last measuring date ($F_{1,\ 120} = 7.64$, $P = 0.007$, Fig. 4). No differences in ARA between the climate change field treatments were found.

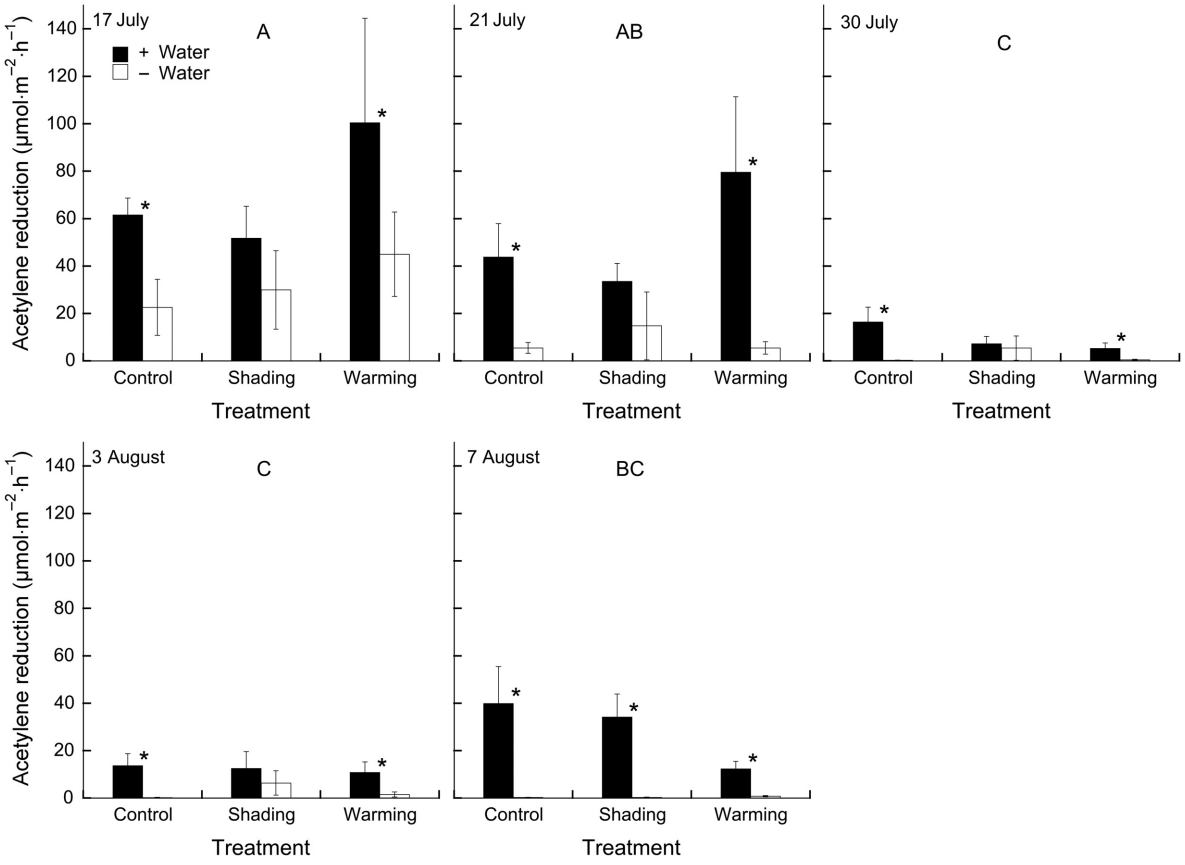

Fig. 4. Acetylene reduction (μmol·m^{-2}·h^{-1}) in three field treatments (control, shading, and warming) from July to August in high arctic tundra, NE Greenland. Shown are mean rates ± SE (n = 5) in cores that received water (black bars) and in cores that did not receive water prior to measurements (white bars). Asterisks indicate significant differences between the treatments with and without added water. Capital letters indicate significant differences in acetylene reduction between dates.

The moisture and temperature dependence of N_2 fixation

Moisture content in the field did not change throughout the season, and soil moisture was higher in the shading plots when no water was added compared to the other plots ($F_{2,11}$ = 1.44, P = 0.007, Fig. 2a; Appendix S1: Fig. S3A). The water additions lead to increased moisture content in all treatments ($F_{2,11}$ = 1.90, P < 0.0001, Fig. 2a). Nitrogen fixation (ARA) increased with increasing moisture content in all climate change field treatments (R^2 = 0.31, P < 0.0001 for all measuring campaigns and treatments, Fig. 5). In the warming treatment, the added water and the moisture content further interacted to influence N_2 fixation (P = 0.002, t = −2.2, Fig. 5).

The climate change field treatments lead to differences in the soil temperature: The lowest soil temperatures were recorded in the shading treatment and the highest in the warming treatment (averaged across the season and water treatments: 12.3 ± 0.02°, 11.0 ± 0.1°, 14.0 ± 0.5°C for the control, shading, and warming plots, respectively, $F_{2,\ 118}$ = 37.52, P < 0.0001, Fig. 2b; Appendix S1: Fig. S3B). The water additions did not lead to changes in soil temperature between the field treatments (Fig. 2b). The lowest temperatures were recorded at the first two sampling time points in July ($F_{4,\ 118}$ = 30.15, P < 0.001, Appendix S1: Fig. S3B).

Acetylene reduction decreased with increasing temperature in all treatments (control, R^2 = 0.27,

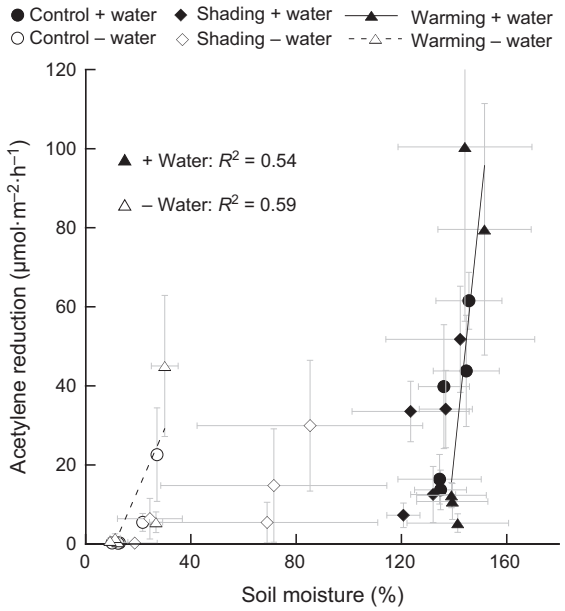

Fig. 5. Acetylene reduction (μmol·m^{-2}·h^{-1}) in relation to gravimetric moisture content (%) in the climate change field treatments control, shading, and warming, in cores with water added (black symbols) and without water added (white symbols). Shown are the rates from the five measuring campaigns during the growing season in high arctic tundra, NE Greenland. Significant relationships are indicated with fitted lines and R^2 for the warming treatment. The other two treatments did not show a significant interaction between acetylene reduction, soil moisture, and added water.

P = 0.0005; shading, R^2 = 0.11, P = 0.03; warming, R^2 = 0.40, P < 0.0001), and no differences in the temperature relationship were found between the treatments with added water and without added water, except in the warming plots (P = 0.007, t = −2.9, Fig. 6).

In the control plots, soil moisture was the most important factor affecting ARA, irrespective of adding water or not (P < 0.0001, R^2 = 0.41). In the shading plots, the added water seems to be the most important driver for ARA, followed by the interactive effects of the added water and soil moisture and soil temperature, and the interactive effects of all three factors (P = 0.007, R^2 = 0.23). In the warming plots, the pattern was similar. The most important factor influencing ARA was the added water, followed by the interactive effect of the added water and soil moisture, and the added water and soil temperature. When the model was run without the added water as factor, soil moisture was the most important factor controlling ARA in all treatments ($P_{control}$ < 0.0001, R^2 = 0.41, $P_{shading}$ = 0.008, R^2 = 0.15, $P_{warming}$ = 0.0001, R^2 = 0.34). In addition, in the warming treatment, the interaction between soil temperature and soil moisture was also significantly affecting ARA (P = 0.0001, t = −4.29).

Soil microbial and chemical characteristics

The soil had low total C and N concentration, and low amounts of available N and P. The high microbial C/N ratio pointed toward fungal dominance of the microbial community. No significant differences were found between the climate change field treatments (control, shading, warming) and between the water additions and no water addition treatments in any of the measured soil parameters (Table 1). Total C and N were higher in the top part of the ARA-cores that

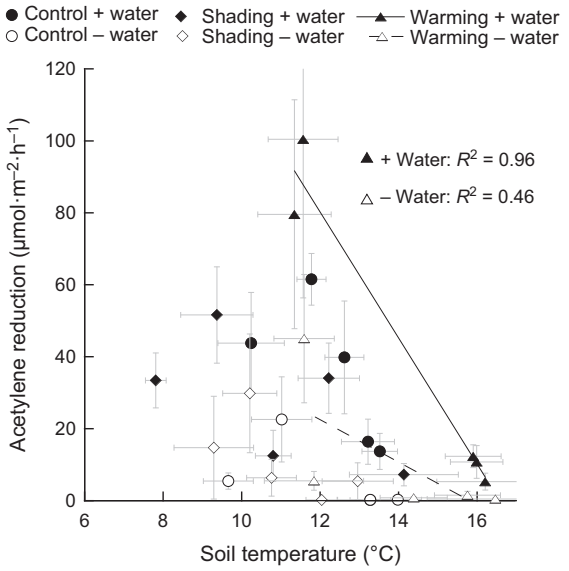

Fig. 6. Acetylene reduction (μmol·m^{-2}·h^{-1}; mean ± SE) in relation to soil temperature in the climate change field treatments control, shading, and warming, in cores with water added (black symbols) and without water added (white symbols). Shown are the rates from the five measuring campaigns during the growing season in high arctic tundra, NE Greenland. Significant relationships are indicated with fitted lines and R^2 for the warming treatment. The other two treatments did not show a significant interaction between acetylene reduction, soil temperature, and added water.

Table 1. Soil microbial and chemical characteristics in the climate change field treatments control, shading, and warming in high arctic tundra, NE Greenland.

Soil characteristics	Control	Shading	Warming
Microbial C (mg g/dw)	0.32 ± 0.11	0.26 ± 0.16	0.17 ± 0.04
Microbial N (mg g/dw)	0.03 ± 0.009	0.02 ± 0.01	0.01 ± 0.004
Microbial C:N	16.39 ± 2.4	10.10 ± 2.2	13.83 ± 0.8
TDN (μg g/dw)	1.9 ± 0.5	2.1 ± 0.4	1.6 ± 0.4
DON (μg g/dw)	1.7 ± 0.5	1.9 ± 0.4	1.3 ± 0.4
DOC (μg g/dw)	34.2 ± 4.4	33.1 ± 4.5	28.3 ± 3.1
Soil C (%)	0.88 ± 0.26	1.09 ± 0.15	0.77 ± 0.22
Soil N (%)	0.06 ± 0.02	0.07 ± 0.01	0.06 ± 0.01
Soil C:N	15.57 ± 0.66	16.11 ± 1.33	14.96 ± 0.50
Total soil $\delta^{15}N$ (‰)	3.29 ± 1.4	6.12 ± 0.24	4.97 ± 0.65
Total soil $\delta^{13}C$ (‰)	−24.83 ± 0.18	−25.38 ± 0.57	−25.43 ± 0.51
NH_4^+ (μg g/dw)	0.13 ± 0.02	0.14 ± 0.05	0.2 ± 0.08
NO_3^- (μg g/dw)	0.13 ± 0.02	0.12 ± 0.02	0.10 ± 0.008
PO_4 (μg g/dw)	0.17 ± 0.04	0.15 ± 0.03	0.10 ± 0.01

Note: Given are means (n = 5) ± SE.

included organic crust and plant material (mean across all field and water treatments: (C: 7.21 ± 1.0% and N: 0.40 ± 0.04%) than in the lower part that included roots (C: 3.21 ± 0.3% and N: 0.20 ± 0.03%), $F_{1,58}$ = 15.3, P = 0.0002, $F_{1,58}$ = 16.6, P = 0.0001 for C and N, respectively).

Discussion

Nitrogen fixation increased with moisture content in all climate change field treatments and was even higher in the warming treatment when water was added. Our analyses also reveal that moisture is more important than temperature for N_2 fixation activity in our study system. Hence, also in the high Arctic, biological N_2 fixation is controlled in a hierarchical way, with moisture being the most important factor for nitrogenase activity, followed by temperature. This confirms previous findings for free-living (Belnap 2001) and moss-associated diazotrophs in the Subarctic (Rousk and Michelsen 2017) and high Arctic (also reviewed in Stewart et al. 2014). For instance, in *Sphagnum* moss-dominated habitats in the Arctic, moisture content explained at least 50% of the variation in N_2 fixation rates in the field (Stewart et al. 2011). Nonetheless, moisture content is an indirect effect of temperature, lichens, mosses and free-living diazotrophs dry out quickly when temperatures increase. Thus, temperature and moisture interact strongly to affect N_2 fixation (Stewart et al. 2011, Rousk et al. 2017*b*). Further, while moisture affects the immediate activity of diazotrophs, temperature shapes the bacterial community composition over long time periods (see, e.g., Rinnan et al. 2009), thereby driving N_2 fixation rates.

In our study, the highest N_2 fixation activity was found at temperatures below 14°C. This stands in sharp contrast to previous findings on the temperature dependence of N_2 fixation, with a temperature optimum around 25°C for symbiotic as well as moss-associated N_2 fixation (Smith 1984, Houlton et al. 2008). Then again, most previous findings are from ecosystems outside the Arctic. Abiotic conditions, such as air temperature can be extreme in arctic habitats, and plant and microbial communities are adapted to those conditions (Billings and Mooney 1968, Bliss 1971, Robinson 2001, Rinnan et al. 2009). Organisms adapted to low temperatures, as found in the Arctic, are also more tolerant to still colder temperatures and can maintain high activity at temperatures that are too low for warm-adapted organisms (Rinnan et al. 2009, Birgander et al. 2013). Free-living cyanobacteria fix N_2 and photosynthesize at low temperatures in Antarctic and Arctic settings (−4°C, Davey 1983, Liengen 1999), and achieve light saturation at low light levels and can fix C (and N_2) when shaded by clouds and potentially by vegetation (Zielke et al. 2002).

Our results further show that, although higher moisture content promoted N_2 fixation, if the soil is already very moist, additional moisture does not necessarily increase N_2 fixation more, as seen

in the shading treatments (Fig. 5). Soil temperatures in the shaded plots were lower than in the other treatments, and more moisture did not increase N_2 fixation in these plots. This indicates that the long-term exposure to lower temperatures might have shifted the diazotroph communities in these plots, adapted to decreased temperatures. This suggests that the long-term exposure to (lower) temperatures drives N_2 fixation. Further, N_2 fixation activity was highest in the warming plots, hinting to a shift in the diazotroph community adapted to higher temperatures (Rousk and Michelsen 2017). This is also reflected in the maximum N_2 fixation activity found above 12°C in the warming, around 12°C in the control, and below 10°C in the shaded plots. These temperature optima are much lower than previously reported. For instance, Hobara et al. (2006) report increasing N_2 fixation rates by free-living and lichen-associated diazotrophs in high arctic tundra up to 30°C, and maximum temperature for N_2 fixation in mosses from the Antarctic have been found between 25° and 27°C (Smith 1984). On the other hand, N_2 fixation rates in different legume species in the high Arctic differ in their temperature optimum ranging between 10° and 25°C (Schulman et al. 1988). In mosses from boreal forests, temperature optima of 13° and 22°C have been reported (Gentili et al. 2005), which was attributed to different cyanobacterial communities with different temperature optima, thereby occupying separate niches on mosses. However, we cannot rule out that N_2 fixation in our study system might be even higher at higher temperatures.

A negative relationship between N_2 fixation and air temperature has been reported previously for lichens and mosses (Rousk et al. 2015). This was ascribed to the drying-out effect of the diazotroph associations by increased temperature. The large range in temperature optimum reported previously (e.g., Smith 1984, Hobara et al. 2006) might have been recorded while the N_2 fixer associations have been optimally moist. Further, mosses differ in their capacity to hold water (Elumeeva et al. 2011), which might also explain differences in N_2 fixation activity between studies investigating different moss species.

Our study further highlights the large differences between measured N_2 fixation rates in situ, and potential N_2 fixation under optimal (moisture) conditions (Fig. 4). Field measurements likely underestimate N_2 fixation rates and should take into account the abiotic conditions prevailing during and shortly before measurements. Most laboratory studies assess N_2 fixation under optimal conditions, making direct comparisons between field and laboratory based measurements ambiguous.

The climate change field treatments did not translate into differences in vegetation cover and plant community composition. This could explain the lack of a treatment effect on N_2 fixation rates, which stands in contrast to our hypotheses. If mosses and other N_2 fixing associations (lichens, free-living diazotrophs) are not outcompeted by faster growing vascular plants, then N_2 fixation rates may remain similar. The absence of a treatment effect on plant coverage is consistent with a recent synthesis showing that some plant communities in high arctic ecosystems respond little to moderate warming (1–3°C, Elmendorf et al. 2012). It is also consistent with unchanged relative growth rate and gross ecosystem production in warmed plots compared to controls in the willow heath at Zackenberg investigated in the current study (Campioli et al. 2013) and with the unchanged normalized differential vegetation index in the same warmed or shaded plots (data from mid-August 2012, not shown). Along those lines, the climate change field treatments did not result in differences in a range of soil measures after 6 yr of initiation of the treatments (Table 1). While small additions of N (0.5 $g \cdot m^{-2} \cdot yr^{-1}$) can lead to significant and rapid (2 yr after N application) shifts in vegetation composition in the high Arctic (Arens et al. 2008), our climate change field treatments did not alter soil N pools. Changes in vegetation composition, N_2 fixation rates, soil N pools, etc., might emerge after a longer period of initiation of field treatments. For instance, an increase in shrub cover at the expense of mosses and lichens has been observed in longer term warming experiments (5–10 yr, Graglia et al. 2001, Hollister et al. 2005, Wahren et al. 2005), and a reduction in moss-associated N_2 fixation has been found in shaded plots in the Subarctic 21 yr after initiation of the field treatments (Sorensen et al. 2012).

Conclusions

Biological N_2 fixation in the high Arctic is strongly affected by moisture conditions. Thus, the

consequences of changing precipitation patterns in a future climate will depend on the prevailing moisture conditions. Further, the diazotroph communities in arctic habitats are adapted to the historically low temperatures and will likely shift in a warmer, future climate. Increasing temperatures will have direct effects on N_2 fixation as well as indirect, long-term effects via shrub expansion into high arctic ecosystems at the expense of lichens, mosses, and free-living diazotrophs. Indirect effects of increased temperatures will likely reduce ecosystem N input via associative N_2 fixation in the long term. However, the net effects of climate change on N_2 fixation are ambiguous and will depend strongly on the degree and interactive effects of temperature, precipitation, and shrub expansion.

Acknowledgments

Funding was provided by the Danish Council for Independent Research "Research Project 1" (Grant ID: DFF—6108-00089), the Carlsberg Foundation (Grant ID: 2009-01-0570) and the Danish National Research Foundation (Center for Permafrost, CENPERM DNRF100). We thank Jannik Hansen, Lars Holst Hansen, and Niels Martin Schmidt for assistance at Zackenberg Research Station and Gosha Sylvester for assistance with laboratory analyses at the University of Copenhagen. Climate data were provided by the Greenland Ecosystem Monitoring Programme.

Literature Cited

Arens, S. J. T., P. F. Sullivan, and J. M. Welker. 2008. Nonlinear responses to nitrogen and strong interactions with nitrogen and phosphorus additions drastically alter the structure and function of a high arctic ecosystems. Journal of Geophysical Research 113. https://doi.org/10.1029/2007JG000508

Belnap, J. 2001. Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. Pages 241–261 *in* J. Belnap and O. L. Lange, editors. Biological soil crusts: structure, function, and management. Ecological Studies (150), Springer, Heidelberg, Germany.

Billings, W. D., and H. A. Mooney. 1968. The ecology of Arctic and Alpine plants. Biological Reviews 43:481–529.

Bintanja, R., and O. Andry. 2017. Towards a rain-dominated Arctic. Nature Climate Change 13:263–268.

Birgander, J., S. Reischke, D. L. Jones, and J. Rousk. 2013. Temperature adaptation of bacterial growth and ^{14}C-glucose mineralisation in a laboratory study. Soil Biology and Biochemistry 65:294–303.

Bliss, L. C. 1971. Arctic and Alpine plant life cycles. Annual Review of Ecology and Systematics 2: 405–438.

Campioli, M., N. M. Schmidt, K. R. Albert, N. Leblans, H. Ro-Poulsen, and A. Michelsen. 2013. Does warming affect growth rate and biomass production of shrubs in the High Arctic? Plant Ecology 214:1049–1058.

Davey, A. 1983. Effects of abiotic factors on nitrogen fixation by blue-green algae in Antarctica. Polar Biology 2:95–100.

Elberling, B., A. Michelsen, C. Schädel, E. A. G. Schuur, H. H. Christiansen, L. Berg, M. P. Tamstorf, and C. Sigsgaard. 2013. Long-term CO_2 production following permafrost thaw. Nature Climate Change 3:890–894.

Ellebjerg, S. M., M. Tamstorf, L. Illeris, A. Michelsen, and B. U. Hansen. 2008. Interannual variability and controls of plant phenology and productivity at Zackenberg. Pages 249–273 *in* H. Meltofte, T. R. Christensen, B. Elberling, M. C. Forchhammer, and M. Rasch, editors. High-arctic ecosystem dynamics in a changing climate. Advances in Ecological Research (40), Elsevier, New York, New York, USA.

Elmendorf, S. C., et al. 2012. Global assessment of experimental climate warming on tundra vegetation: heterogeneity over space and time. Ecology Letters 15:164–175.

Elumeeva, T. G., N. A. Soudzilovskaia, H. J. During, and J. H. C. Cornelissen. 2011. The importance of colony structure versus shoot morphology for the water balance of 22 subarctic bryophyte species. Journal of Vegetation Science 22:152–164.

Gavazov, K. S., N. A. Soudzilovskaia, R. S. P. van Logtestijn, M. Braster, and J. H. C. Cornelissen. 2010. Isotopic analysis of cyanobacterial nitrogen fixation associated with subarctic lichen and bryophyte species. Plant and Soil 333:507–517.

Gentili, F., M. C. Nilsson, O. Zackrisson, T. H. DeLuca, and A. Sellstedt. 2005. Physiological and molecular diversity of feather moss associative N_2-fixing cyanobacteria. Journal of Experimental Botany 56: 3121–3127.

Graglia, E., S. Jonasson, A. Michelsen, I. K. Schmidt, M. Havström, and L. Gustavson. 2001. Effects of environmental perturbations on abundance of subarctic plants after three, seven and ten years of treatments. Ecography 24:5–12.

Gundale, M. J., M. Nilsson, S. Bansal, and A. Jäderlund. 2012. The interactive effects of temperature and light on biological nitrogen fixation in boreal forests. New Phytologist 194:453–463.

Hartley, I. P., M. H. Garnett, M. Sommerkorn, D. W. Hopkins, B. J. Fletcher, V. L. Sloan, G. K. Phoenix, and P. A. Wookey. 2012. A potential loss of carbon associated with greater plant growth in the European Arctic. Nature Climate Change 2:875–879.

Hobara, S., C. McCalley, K. Koba, A. E. Giblin, M. S. Weiss, G. M. Gettel, and G. R. Shaver. 2006. Nitrogen fixation in surface soils and vegetation in an arctic tundra watershed: a key source of atmospheric nitrogen. Arctic Antarctic and Alpine Research 3:363–372.

Hollister, R. D., P. A. Webber, and C. E. Tweedie. 2005. The response of Alaskan tundra to experimental warming: differences between short-and long-term responses. Global Change Biology 11:525–536.

Houlton, B. Z., Y. P. Wang, P. M. Vitousek, and C. B. Field. 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454:327–331.

Intergovernmental Panel on Climate Change (IPCC). 2013. Climate change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, edited by T. F. Stocker et al., Cambridge University Press, Cambridge, UK.

Jensen, L. M., and M. Rasch. 2011. Zackenberg ecological research operations, 16th Annual Report, 2010. Aarhus University, DCE – Danish Centre for Environment and Energy, Aarhus, Denmark.

Jensen, L. M., E. Topp-Jørgensen, T. R. Christensen, and N. M. Schmidt. 2016. Zackenberg ecological research operations 20th Annual Report, 2014. Aarhus University, DCE – Danish Centre for Environment and Energy, Aarhus, Denmark.

Jonasson, S. 1983. The point intercept method for nondestructive estimation of biomass. Phytocoenologia 11:385–388.

Keuper, F., P. M. van Bodegom, E. Dorrepal, J. T. Weeden, J. van Hal, R. S. P. van Logtestijn, and R. Aerts. 2012. A frozen feast: Thawing permafrost increases plant-available nitrogen in subarctic peatlands. Global Change Biology 18:1998–2007.

Liengen, T. 1999. 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.

Michelsen, A., R. Rinnan, and S. Jonasson. 2012. Two decades of experimental manipulations of heaths and forest understory in the subarctic. Ambio Supplement 3, 41:218–230.

Peñuelas, J., et al. 2013. Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. Nature Communications 4:2934–2944.

R Developmental Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rinnan, R., J. Rousk, E. Yergeau, G. A. Kowalchuk, and E. Bååth. 2009. Temperature adaptation of soil bacterial communities along an Antarctic climate gradient: predicting responses to climate warming. Global Change Biology 15:2615–2625.

Robinson, C. H. 2001. Cold adaptation in Arctic and Antarctic fungi. New Phytologist 151:341–353.

Rousk, K., J. Degboe, A. Michelsen, R. Bradley, and J. P. Bellenger. 2017*a*. Molybdenum and phosphorus limitation of moss-associated nitrogen fixation in boreal ecosystems. New Phytologist 214:97–107.

Rousk, K., P. A. Pedersen, K. Dyrnum, and A. Michelsen. 2017*b*. The interactive effects of temperature and moisture on nitrogen fixation in two temperate-arctic mosses. Theoretical and Experimental Plant Physiology 29:25–36.

Rousk, K., D. L. Jones, and T. H. DeLuca. 2014. The resilience of nitrogen fixation in feather moss (*Pleurozium schreberi*)-cyanobacteria associations after a drying and rewetting cycle. Plant and Soil 374:513–521.

Rousk, K., and A. Michelsen. 2017. Ecosystem nitrogen fixation throughout the snow-free period in subarctic tundra: effects of willow and birch litter addition and warming. Global Change Biology 23:1552–1563.

Rousk, K., J. Rousk, D. L. Jones, O. Zackrisson, and T. H. DeLuca. 2013. Feather moss nitrogen acquisition across natural fertility gradients in boreal forests. Soil Biology and Biochemistry 61:86–95.

Rousk, K., P. L. Sorensen, S. Lett, and A. Michelsen. 2015. Across habitat comparison of diazotroph activity in the Subarctic. Microbial Ecology 69:778–787.

Rousk, K., P. L. Sorensen, and A. Michelsen. 2016. Nitrogen transfer from four nitrogen fixer associations to plants and soils. Ecosystems 19:1491–1504.

Schulman, H. M., M. C. Lewis, E. M. Tipping, and L. M. Bordeleau. 1988. Nitrogen fixation by three species of *leguminosae* in the Canadian High Arctic Tundra. Plant, Cell and Environment 11:721–728.

Schuur, E. A. G., et al. 2015. Climate change and the permafrost carbon feedback. Nature 520:171–179.

Seddon, A. W. R., M. Macias-Fauria, P. R. Long, D. Benz, and K. J. Willis. 2016. Sensitivity of global terrestrial ecosystems to climate variability. Nature 531:229–232.

Shaver, G. R., and F. S. Chapin III. 1980. Response 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

subantarctic island. Applied and Environmental Microbiology 48:594–600.

Sorensen, P. L., S. Lett, and A. Michelsen. 2012. Moss-specific changes in nitrogen fixation following two decades of warming, shading, and fertilizer addition. Plant Ecology 213:695–706.

Sorensen, P. L., A. Michelsen, and S. Jonasson. 2008. Ecosystem partitioning of ^{15}N-glycine after long-term climate and nutrient manipulations, plant clipping and addition of labile carbon in a subarctic heath tundra. Soil Biology and Biochemistry 40: 2344–2350.

Stewart, K. J., D. S. Coxson, and P. Grogan. 2011. Nitrogen inputs by associative cyanobacteria across a low arctic tundra landscape. Arctic, Antarctic and Alpine Research 43:267–278.

Stewart, K. J., P. Grogan, D. S. Coxson, and S. D. Siciliano. 2014. Topography as a key factor driving atmospheric nitrogen exchanges in arctic terrestrial ecosystems. Soil Biology and Biochemistry 70: 96–112.

Taylor, B. N., R. L. Chazdon, B. Bachelot, and D. N. L. Menge. 2017. Nitrogen-fixing trees inhibit growth of regenerating Costa Rican rainforests. Proceedings of the National Academy of Sciences USA 114:8817–8822.

Turetsky, M. R. 2003. The role of bryophytes in carbon and nitrogen cycling. Bryologist 106:395–409.

Van Cleve, K., and V. Alexander. 1981. Nitrogen cycling in tundra and boreal ecosystems. Pages 375–404 *in* F. E. Clark and T. Rosswall, editors. Terrestrial nitrogen cycles. Ecological bulletins. Swedish Natural Science Research Council (33), Stockholm, Sweden.

Van Cleve, K., L. K. Oliver, P. Schlentner, L. A. Viereck, and C. T. Dyrness. 1983. Productivity and nutrient cycling in taiga forest ecosystems. Canadian Journal of Forest Research 13:747–766.

Wahren, C. H. A., M. D. Walker, and M. S. Bret-Harte. 2005. Vegetation responses in Alaskan arctic tundra after 8 years of a summer warming and winter snow manipulation experiment. Global Change Biology 11:37–552.

Zielke, M., A. S. Ekker, R. A. Olsen, S. Spjelkavik, and B. Solheim. 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., B. Solheim, S. Spjelkavik, and R. A. Olsen. 2005. Nitrogen fixation in the high arctic: role of vegetation and environmental conditions. Arctic, Antarctic and Alpine Research 37:372–378.

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