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Bryophyte species differ widely in their growth and N₂-fixation responses to temperature

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Abstract

Bryophytes are abundant in tundra ecosystems, where they affect carbon and nitrogen cycling through primary production and associations with N₂-fixing bacteria. Bryophyte responses to climate warming are inconclusive, likely because species-specific responses are poorly understood. Here we investigated how warming affects the growth and nitrogenase activity of 10 tundra bryophyte species in two tundra landscapes. Collected bryophyte samples were grown in temperature-controlled growth chambers for 12 weeks at five temperatures from 3 to 18 °C. We measured growth, N concentration, δ¹⁵N, and δ¹³C after 3 months and nitrogenase activity after 5 and 12 weeks. Bryophyte growth and associated nitrogenase activity generally increased with temperature, but species differed in their optima. Bryophyte N concentration and δ¹⁵N indicated that, for some species, increased N₂-fixation could not compensate for growth-induced N limitation. High landscape coverage and large positive warming effects on feather mosses and Sphagnum species highlight their competitive advantages, confirm earlier field observations, and contribute to the mechanistic understanding of differential bryophyte growth in response to warming. We suggest that indirect effects of climate change, such as surface drying and shrub expansion, are likely main threats to slow-growing bryophytes across the Arctic, with consequences for biodiversity and C balance.

Key words: acetylene reduction assay, δ¹³C, δ¹⁵N, mosses, nitrogen cycling, subarctic tundra ecosystems

Introduction

The temperature in the Arctic regions is increasing at a rate more than twice the global average (IPCC 2018). Elevated temperatures enhance microbial decomposition and increase nutrient availability in usually nutrient-poor soils (Voigt et al. 2017). Increased temperatures and nutrient availability generally decrease bryophyte diversity and abundance, as bryophytes are outcompeted by vascular plants, but responses are mixed (Wijk et al. 2004; Elmendorf et al. 2012; Alatalo et al. 2020; Zuijlen et al. 2021). Bryophytes play im-
portant roles in tundra ecosystems for primary production (Turetsky et al. 2012) and nutrient cycling (Longton 1997). They also regulate soil temperature and moisture, control nitrogen (N) availability for vascular plants (Cornall et al. 2007), provide habitat and food sources for microbes and invertebrates and promote carbon storage (Turetsky et al. 2012; Hájek 2014). Changes in bryophyte cover and community composition will therefore likely lead to important ecosystem changes (Cornelissen et al. 2007; Deane-Coe and Stanton 2017). However, our predictions of future bryophyte distribution remain poorly resolved because species-specific growth responses of bryophytes to increasing temperature lack mechanistic understanding.

Species diversity of bryophytes is great at high latitudes (Jägerbrand et al. 2006), where variation in phenotypic plasticity allows them, to some extent, to persist in the same place even if habitat conditions change (Aherston et al. 2010; Turetsky et al. 2012; Lonnell and Hallingbäck 2019). The growth of British bryophytes shows clear species differences in temperature optima, and the impact of a certain temperature increase depends on the species niche breadth (i.e., temperature tolerance) and particular optimum (Furness and Grime 1982; Berg and Ellers 2010). Similarly, at a specific location, species with a more southern provenance might respond positively to temperature increases while those typical to higher latitudes might not benefit from higher temperatures (Vandvik et al. 2020). For these reasons, responses of organisms, including bryophytes, to climate change often seem complex and context dependent (Zuijlen et al. 2021). Understanding how tundra bryophytes respond across a spectrum of temperatures, and how these responses vary between species, could help unravel the fate of bryophytes in a rapidly warming Arctic.

Bryophytes are functionally different from vascular plants. They lack roots and take up water and nutrients through their leaves, and access N through deposition (Pitcairn et al. 1995; Zechmeister et al. 2007), from soil and precipitation as inorganic and organic N forms (Ayres et al. 2006; Krab et al. 2008), and directly from the atmosphere through N2-fixing associated bacteria (Gundale et al. 2011). N2-fixation is an enzymatic process carried out by nitrogenase, which typically incorporates molybdenum as a co-factor (Miller and Eady 1988; Nelson et al. 2019), and which has a temperature optimum of around 25 °C (Houltan et al. 2008). Warming should therefore increase N2-fixation in cold ecosystems. However, in nature, warming can have neutral or even negative effects as it often leads to concurrent drying (Gundale et al. 2012b; Stewart et al. 2014; Rousk et al. 2017). Bryophyte species differ in their structure and ability to retain water (Elumeeva et al. 2011; Lett et al. 2021). Species vary in N2-fixation rates (Stuart et al. 2020), but species-specific temperature responses to temperature remain poorly understood.

N2-fixation is the main source of new N in Arctic ecosystems, and it can exceed atmospheric deposition in remote regions, reaching up to 3 kg N ha−1 year−1 (Gundale et al. 2011; Rousk and Michelsen 2017). This bryophyte-associated N could becomeavailable to the ecosystem after disturbance events (e.g., fire events or drying-rewetting), or via mycorrhizal associations and mineralisation (Rousk et al. 2016; DeLuca et al. 2021). However, this N is also crucial for bryophyte growth and thus, carbon capture in the ecosystem (Berg et al. 2013; Vile et al. 2014).

The presence of rare N and C isotopes in mosses can reveal patterns of growth conditions accumulated over time (Deane-Coe et al. 2015). The content of the 15N isotope in bryophyte shoots can indicate the dominant source of N (Pearson et al. 2000; Solga et al. 2005; Power and Collins 2010) because the N forms differ in isotopic signatures. Reduced forms such as NH4+ tend to be depleted, and oxidized forms (NO3−) are enriched with 15N, relative to the atmospheric N pool. Nitrogenase has low discrimination against 15N (Vitousek et al. 1989), and therefore values approaching 0% δ15N could be expected in bryophyte shoots when N2 is the dominant N source (Deane-Coe 2015). However, earlier research is scarce and does not find a significant relationship between δ15N and the quantity of symbiotically fixed N2 (Stuart et al. 2021, Hyodo et al. 2013), although near zero δ15N values indicated high N2 uptake across six Sphagnum species (Leppänen et al. 2015). In a similar manner, bryophyte tissue δ15N can provide insight into relative growth rates and photosynthetic activity of bryophytes (Rice 2000), and it is often used as an indicator of environmental factors, such as moisture and temperature conditions (Rice 2000; Bramley-Alves et al. 2015; Granath et al. 2018). In bryophytes, C uptake is lower at high water content due to low CO2 diffusion through water (Rice and Giles 1996; Williams and Flanagan 1996). The photosynthetic enzyme Rubisco discriminates against the heavy C isotope but will increase 13C uptake when C is limiting (Farquhar et al. 1989). Consequently, hydrated bryophytes covered by a water film have generally higher values of δ13C, while bryophytes growing under dry conditions have lower values (Deane-Coe et al. 2015; Bramley-Alves et al. 2016). While theoretically a strong tool for assessing recent N and C dynamics in mosses, relationships may be species-specific (Granath et al. 2018) and we still need a better understanding of environmental controls on this specificity to clarify the value of these isotopic tools in ecological studies of mosses.

This study aimed to investigate bryophyte species-specific growth and nitrogenase activity responses to a range of temperatures and to understand how these responses relate to N concentration in bryophyte tissues. We assessed bryophyte coverage in two tundra landscapes and set up a growth chamber experiment in which we exposed 10 of the most common bryophyte species to five temperature treatments for 12 weeks. We aimed to maintain equally high moisture across all temperatures to eliminate any drying effect of the temperature treatments. We hypothesised that (1) bryophyte growth and nitrogenase activity responses to increasing temperature will be species specific, where species with a higher water-holding capacity will respond positively to increasing temperature and (2) bryophyte growth will correlate positively with nitrogenase activity. Testing these hypotheses will contribute to our mechanistic understanding of species-specific responses of bryophytes to climate warming and add to our general knowledge of bryophyte functional diversity.
Materials and methods

Site characterisation and bryophyte sample collection

Bryophytes were collected from two subarctic, alpine sites in northern Sweden: Katterjäkk (68°25′08″N, 18°10′05″E) and Vassijaure (68°25′35″N, 18°15′08″E; ~550 m a.s.l., c. 40 and 35 km west of Abisko), with mean annual temperature and precipitation of ~1.7 °C and 843.7 mm. The growing season monthly temperature ranges (1961–1990) are 5–12 °C in June, 7–17 °C in July, and 5–13 °C in August (Swedish Meteorological and Hydrological Institute, SMHI). The sites are nutrient-poor, moss-rich heathland ecosystems dominated by shrubs such as Empetrum nigrum L., Vaccinium sp., Betula nana L., Salix herbaecea L., and Salix lapponum L., and graminoids as Eriophorum angustifolium L., Deschampsia flexuosa Trin. and Carex vaginata Tausch (Carlsson et al. 1999).

We collected bryophyte samples on August 27 (Katterjäkk) and 28 (Vassijaure; Supporting Information 1) 2019 by cutting 200 intact, monospecific bryophyte blocks (11 cm × 11 cm × 10 cm height) and transferring them to plastic pots. The collected species were Sphagnum fuscum (Schimp.) Klinggr., Sphagnum compactum Lam. & DC., Dicranum scoparium Hedw., Hylcomium splendens (Hedw.) Bruch, Schimp. & W.Guembel, Pleurozium schreberi (Brd.) Mitt., Scorpidium revolvens (Sw. ex anon.) Rubers., Ptildium ciliare (L.) Hampe, Barbilophozia floerkei (E.Weber & D.Mohr) Loeske, Racotritium lanuginosum (Hedw.) Brd., and R. fasciculare (Hedw.) Brd.

To quantify the abundance of our selected bryophyte species relative to vegetation including moss cover in general, we selected quadrats of 4 m² located every 100 m along seven (Katterjäkk) and eight (Vassijaure) 2000 m transects in the area of our collected bryophyte samples (Katterjäkk, n = 140; Vassijaure, n = 160). Specifically, we estimated the cover inside the quadrats of bare rock, lichens, herbageous plants, shrubs, total bryophyte, and specific, collected bryophyte species.

Preparation of mesocosms and experimental setup

Bryophyte material was kept in the shade outside the Abisko Scientific Research station during collection and was packed securely in boxes to be brought by plane to the University of Copenhagen within approximately 6 h. Here they were placed directly in climate-controlled growth chambers. Bryophyte colony height was modified by cutting from below to standardise the organic soil layer underneath the colony to 2–4 cm, depending on species, or specifically to 0.5 cm in R. lanuginosum, which had a very thin layer of soil. Within-species variation in colonies’ height was 0.5 cm. Sphagnum spp. had no organic soil and were therefore cut to a standard depth of 5–6 cm. Each pot (11 × 11 × 11 cm) contained a paper filter at the bottom, sand (grain size 0–2 mm), and a bryophyte colony placed on top. Since bryophyte replicates differed in height, the amount of sand varied, and the final height of a sample unit was 10 cm with 1 cm of headspace. A few non-target species (including N₂-fixing lichens) were removed from the colonies.

A total number of 200 pots were distributed between five independent climate chambers so that four replicate pots of each bryophyte species were placed in each chamber. Climate chambers are custom made from chest freezers (60 × 150 × 86 cm height) with eight fluorescent light tubes installed over the transparent lid and with small holes near the bottom to allow for air exchange. Air is moved inside the chamber with fans, and temperature is controlled via thermostats connected to a computer. Bryophyte pots were left to settle for 15 days at 3 °C. On 17 September, the experiment was started, with five temperature treatments. The goal was to create a gradient with a difference of 3–5 °C between each chamber. Temperatures were continuously monitored, and the achieved mean temperatures were 3, 6, 9, 13, and 18 °C, and approaching the range of mean minimum to mean maximum temperatures in the Abisko region during the growing season (SMHI). We changed pot placement four times during the experiment between chambers (with the temperature setting assigned to specific pots remaining the same) to prevent a “chamber effect.” During the full period, each chamber had light sources providing 24 h of daylight with 250 μmol m⁻² s⁻¹ in the Photosynthetic Active Radiation (PAR) range to imitate cloudy summer conditions in subarctic Sweden, in the period from late May to late July. The constant moisture level was maintained by spraying until full saturation 6 days a week with distilled water. The experiment was terminated 12 weeks after initiating the temperature treatment.

Bryophyte growth

Bryophyte growth was measured either as shoot length increment or as colony height, depending on the particular growth form of the bryophyte species. We measured shoot length increment on H. splendens, Scorpidium revolvens, Ptildium ciliare, Pleurozium schreberi, R. lanuginosum, and R. fasciculare, which are all branching bryophyte species that do not necessarily grow vertically. Here we tied a cotton thread to two shoots per pot, and the distance from the shoot tip to the thread knot was measured immediately, and again after 64 days (9 weeks). The difference between the two lengths was calculated (modified white marks method from Pouliot et al. 2010). Although not always recommended for small-statured bryophytes (Russell 1988), we measured colony height increment of Barbilophozia floerkei and Dicranum scoparium, and Sphagnum spp. with the cranked wire method (Clymo 1970), by tying the cotton thread resulted in damage to their fragile shoots. This method is standard for the Sphagnum mosses (Clymo 1970) and was also considered to be the most appropriate method for Barbilophozia floerkei and Dicranum scoparium which both have vertical growth. Specifically, a plastic stick was carefully inserted through the bryophyte colony to the bottom of each pot and marked at the surface of the bryophyte after 0 and 63 days. Colony height increment was the distance between the two markings.

Nitrogenase activity

Nitrogenase activity was measured with the acetylene reduction assay, which measures the activity of enzyme nitrogenase (Rousk and Michelsen 2017). Acetylene reduction was
measured after 5 and 12 weeks of temperature treatment to reflect short-term, direct effects, and long-term, direct and indirect effects combined, respectively. Indirect effects include effects on bacteria and bryophyte growth and bacterial community changes. On each measurement day, shoots including both green and brown biomass were collected from an area of 3.5 cm² from each pot, placed in a glass vial (20 mL volume), and sprinkled with distilled water to full saturation. If necessary, shoots were clipped from the bottom to fit the 75 mm height of the vial. Each vial was sealed with a perforated parafilm and placed in the corresponding climate chamber until the following day (Supporting Information 1). The following day, 10% of the headspace was replaced with acetylene (98%). Vials were incubated in the corresponding climate chamber for 24 h, after which 6 mL of gas was sampled in pre-evacuated vials (Labco® exetainers) to analyse ethylene concentrations on a gas chromatograph (SRI310C, FID, SRI Instruments, Torrance, CA, USA). Blank samples without bryophyte shoots were included to correct for background ethylene concentrations. After incubation, shoots were dried, and nitrogenase activity rates were calculated per square meter per hour.

Water-holding capacity
Bryophyte water-holding capacity was measured at the end of the experiment. An area of 12.5 cm² of bryophyte was sampled and rinsed from sand in water, water-saturated, weighed, dried for 48 h at 85 °C, and weighed again. Water-holding capacity was expressed as % water of bryophyte dry weight.

Bryophyte tissue N concentration, δ¹⁵N and δ¹³C
To measure initial tissue N concentrations, δ¹⁵N, and δ¹³C, a subsample of bryophyte shoots from an area of approximately 3.5 cm² was collected from each pot in the 3 °C chamber on 25 September. We assume these values represent bryophyte element composition under field conditions. To measure tissue element composition after 12 weeks of temperature treatment, and to be able to relate the results to nitrogenase activity, we used bryophyte shoots collected for the acetylene reduction assay across all temperature treatments. We divided shoots into shoot tip and brown part. For other species, the growth differences between the temperature treatments to be insignificant. Finally, we correlated shoot tip N concentration and nitrogenase activity, δ¹⁵N and nitrogenase activity, and bryophyte growth and nitrogenase activity using Pearson’s test. We used an α level of 0.05 as a threshold for significance in all analyses. If necessary, log and square root transformations were made on the response variables to meet the assumptions of normality and homoscedasticity (Table 1).

Results
Bryophyte coverage in the tundra landscape
The total bryophyte cover was 37% and 42% in the two tundra sites (Fig. 1). In both sites, Ptilidium ciliare, Pleurozium schreberi, Dicranum sp., Barbilophozia sp., and H. splendens were the most common species. Together these five species covered 18% and 22% of the ground and made up 42% and 59% of the total bryophyte cover. Sphagnum fuscum, Sphagnum compactum, and R. fascicular were the least common species. Together these three species covered 0.4% and 0.5% of the ground and made up 1% of the bryophyte cover. The vascular plant cover was 32% and 42%, and the lichen cover was 11% and 16%. Bare rock covered 5% and 25% of the ground.

Water-holding capacity
The two Sphagnum species had by far the highest water-holding capacity (1600% to over 1800% of dry weight) compared to the other bryophyte species. Racomitrium lanuginosum and R. fascicular had the lowest water-holding capacity, but only around 15% lower than the other six species (not always significant). Their water-holding capacity was around 630%–830% of dry weight and did not differ from each other (Table 2).

Bryophyte growth
Growth, measured either as shoot length growth or colony height change, differed greatly between bryophyte species and was highest in Sphagnum compactum with the average of 7.9 ± 1.3 mm. This was tenfold greater than in R. lanuginosum with the lowest average growth across all temperatures (Fig. S2, right). Higher temperatures generally increased bryophyte growth, although some species did not respond or showed complex responses to increasing temperature (Table 1, Fig. 2). As such, Sphagnum fuscum and Sphagnum compactum had the largest growth response to increasing temperature. For Sphagnum compactum, Barbilophozia floerkei, Ptilidium ciliare, and Scorpidium revolvens growth declined in the warmest treatment after peaking at 13 °C, although not always significantly. Growth of Dicranum scoparium and R. fascicular generally declined in response to increasing temperature, although Dicranum scoparium did not grow much in the coldest temperature treatment. Pleurozium schreberi seemed to respond positively to increasing temperature, but high variation caused differences between temperature treatments to be insignificant. For other species, the growth differences between the treatments were not significant.

Statistical methods
Data were analysed with R (R Core Team 2019) and Microsoft Excel (Microsoft Corporation 2019). We used a two-way analysis of variance (ANOVA), followed by post hoc Tukey’s tests to test for effects of bryophyte species, temperature treatment, and their interactions on growth, N₂ fixation, water-holding capacity, shoot nitrogen concentration, δ¹⁵N, and δ¹³C. Additionally, we used Dunnett’s test to test the differences in elemental composition (N%, δ¹⁵N, δ¹³C) of species between their natural field conditions and after temperature treatments. We used a one-sample Wilcoxon’s signed rank test to test if the lowest recorded nitrogenase activity is higher than zero. Finally, we correlated shoot tip N concentration and nitrogenase activity, δ¹⁵N and nitrogenase activity, and bryophyte growth and nitrogenase activity using Pearson’s test. We used an α level of 0.05 as a threshold for significance in all analyses. If necessary, log and square root transformations were made on the response variables to meet the assumptions of normality and homoscedasticity (Table 1).
Nitrogenase activity

After 5 weeks of temperature treatment, nitrogenase activity at 3 and 6 °C, as measured with the acetylene reduction assay, was significantly higher than zero (p < 0.001), and around 20% of that in two highest temperatures (SI 3, left). Between species, nitrogenase activity was lowest in *R. lanuginosum* and *Sphagnum fuscum*, which had 90%–97% lower activity than all the other species (Fig. S3, right). Overall, nitrogenase activity responded similarly across bryophyte species to 5 weeks of temperature treatment (with no species × temperature interaction, Table 1; Fig. 3). After another 7 weeks, and a total of 12 weeks of temperature treatment, nitrogenase activity was greatly increased by higher temperature, although the extent of the response depended on bryophyte species (species × temperature interaction; Table 1, Fig. 3b). All species had limited nitrogenase activity at 3 °C and activity peaked at 13 or 18 °C. *Pleurozium schreberi* and *H. splendens* had the highest measured nitrogenase activity and the highest temperature response numerically, although this was not significant for *H. splendens*. For both species, activity peaked at 13 °C, and at 18 °C it dropped drastically to the same level as at 9 °C, less than 20% of maximum activity. *Sphagnum fuscum*, *Ptilidium ciliare*, and *R. lanuginosum* had the lowest average activity, and except for *Sphagnum fuscum*, which responded very moderately, activity did not change with increasing temperature. Nitrogenase activity in *Scorpidium revolvens* and *Dicranum scoparium* was intermediate and only moderately increased by temperature. Nitrogenase activity was positively correlated to bryophyte growth across all the tested species (Table S1).

Bryophyte tissue nitrogen concentration

After 11 weeks, shoot tip N concentration of bryophytes had generally decreased relative to the natural (field) condition. This was most pronounced for *Sphagnum fuscum*, *Sphagnum compactum*, *Scorpidium revolvens*, *Barbilophozia floerkei*, and *R. fasciculare* (Dunnett’s test, Fig. 4). Depending on the species, the N concentration of shoot tips was either unchanged or higher in the warmest treatments. The average N concentration in the shoot tips was highest in *Scorpidium revolvens* (0.83%) and lowest in *R. lanuginosum* (0.31%; Fig. S4, right). Nitrogen concentration peaked at 13 and 18 °C in *Sphagnum fuscum*, *Dicranum scoparium* and *R. fasciculare*, and *H. splendens* and *Pleurozium schreberi* showed similar, but non-significant patterns (Fig. 4). *Sphagnum fuscum*, *Scorpidium revolvens* (NS), *Ptilidium ciliare*, and *Barbilophozia floerkei* had lowest shoot N concentration at 9 °C. *Sphagnum compactum* and *R. lanuginosum* shoot tip N concentrations were not affected by temperature. In contrast to the shoot tips, N concentrations in the brown parts of the shoot generally remained at natural (field) values (Figs. S5 and S6). Finally, N concentration and nitrogenase activity were positively correlated across all species, but the relationship was only marginally significant (p = 0.078; Table S1).

Bryophytes shoot δ15N and δ13C

After 11 weeks of temperature treatment, δ15N in bryophyte shoot tissue increased relative to the natural (field) conditions in three species, *Pleurozium schreberi*, *H. splendens*, and *Dicranum scoparium*. *Racomitrium fasciculare* and *Barbilophozia floerkei* had at some temperatures treatments lower δ15N than at natural (field) conditions, but this did not follow

### Table 1. Two-way ANOVA for effects of temperature, species, and interactions on measured traits across bryophyte species. Bold values indicate a significant effect (p < 0.05).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Temperature</th>
<th>Species</th>
<th>Temperature × Species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Water holding capacity</td>
<td>F</td>
<td>1.5</td>
<td>29.1</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.2</td>
<td>&lt;0.001</td>
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<tr>
<td>Bryophyte length growth</td>
<td>F</td>
<td>9.3</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N2-fixation after 5 weeks</td>
<td>F</td>
<td>8.8</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N2-fixation after 12 weeks</td>
<td>F</td>
<td>22.8</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Shoot tip N concentration</td>
<td>F</td>
<td>11.0</td>
<td>41.8</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Brown (bottom) part N concentration</td>
<td>F</td>
<td>3.0</td>
<td>63.7</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Shoot tip δ15N</td>
<td>F</td>
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<td>73.3</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.8</td>
<td>&lt;0.001</td>
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<tr>
<td>Shoot tip δ13C</td>
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<td>146.2</td>
<td>93.6</td>
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<tr>
<td></td>
<td>p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1Data have been log-transformed.
2Data have been square root transformed.
Fig. 1. Average (±SE) vegetation composition along seven and eight 2 km-long transects in (a) Kåtterjäkk (n = 140) and (c) Vassijaure (n = 160), respectively. The plant cover was estimated visually as % cover in twenty 2 × 2 m² plots per transect. Cover categories are four plant functional types and bare rock. Bryophyte species or genera examined in this study are presented in (b) Kåtterjäkk and (d) Vassijaure.
Nitrogenase activity in response to temperature across bryophyte species

In line with our first hypothesis, we observed generally positive responses of nitrogenase activity to increasing temperatures, which depended on bryophyte species. Although very low, nitrogenase activity took place at 3 and 6 °C (Fig. S3, left). These observations highlight the potential importance of N₂ fixation in ecosystems where low temperatures constrain decomposition and limit the availability of accessible N (Shaver and Chapin 1986; Sistla et al. 2012). All species in our study had similar nitrogenase activity at 3 °C, perhaps partly because moisture is not limiting at low temperatures and species differences in morphology therefore play a less

any specific pattern. In general, the temperature had minimal influence on δ¹³N, and only affected Sphagnum fuscum and Barbilophozia floerkei (Fig. 5). We did not find a significant correlation between bryophyte δ¹⁵N and nitrogenase activity (Table S1).

The 13 and 18 °C treatments consistently decreased δ¹³C in all species relative to the three coldest temperature treatments, which were unchanged from the natural (field) condition (Fig. 6 and Fig. S7, left).

Discussion

Bryophytes are important ecosystem components across tundra habitats, yet their responses to climate warming in these ecosystems are not well understood (Elmendorf et al. 2012; Lang et al. 2012; Lewis et al. 2017). Here we show that 10 common bryophyte species vary substantially in their growth and associated nitrogenase activity responses after 3 months at different temperatures in climate chambers. Below we discuss species-specific temperature responses to warming, links between growth and N dynamics, and the potentially important implications for bryophyte-dominated community responses to climate change.

Bryophyte growth responses to temperature

Bryophyte growth generally responded to temperature, but responses were species-specific and often nonlinear. In agreement with our first hypothesis, bryophyte species that were better at retaining water, namely the two Sphagnum species, grew more and responded more positively to higher temperatures. Although historical habitat characteristics, such as water and nutrient availability, inevitably limit bryophyte growth and result in inter-specific variation, the intraspecific growth variation is controlled mostly by current climatic conditions of the site (Furness and Grime 1982; Zechmeister 1995). The over 10-fold increase in Sphagnum growth under the temperature increase recorded in our study is notably more than observed in a natural setting (Dorrepaal et al. 2004). Sphagnum mosses have special water-holding (hyaline) cells and grow in dense colonies which ensures a stable moisture environment and physical support for vertical growth (Bengtsson et al. 2016, 2018). The two common feather mosses H. splendens and Pleurozium schreberi grew less than the Sphagnum species but also benefited from higher temperatures. Feather mosses dry out relatively easily (Elumeeva et al. 2011), yet their positive responses to temperature suggest that they can be active even at low moisture content, as implied by decreased δ¹³C at high temperatures. Positive temperature response agrees with field observations of increasing growth and cover of feather mosses with warming (Callaghan et al. 1997; Lang et al. 2012; Zuijlen et al. 2021). Interestingly, H. splendens grew well even at 3 °C, which is in line with its Arctic-boreal provenance compared to boreo-temperate Pleurozium schreberi (Busby et al. 1978; Lang et al. 2012).

For several species, notably Sphagnum compactum, Ptilidium ciliare, and Dicranum scoparium, growth responses stagnated or decreased at 18 °C. While 18 °C as a 24-h average is an extreme scenario for subarctic tundra, it is not unlikely for temperatures to reach this high or even higher on warm and sunny days (Gagnon et al. 2018, SMHI). Our results indicate that although warming directly promotes the growth of most species tested here, extended periods of high summer temperatures could be harmful. In contrast to vascular plants, bryophytes do not possess well-developed morphological adaptations to control internal water loss, and instead they regulate their physiological responses, e.g., by suspending their metabolism (Proctor et al. 2007; Turetsky et al. 2012). We aimed to provide enough water to the bryophytes, but a decline in δ¹³C revealed that most species experienced drought-like conditions in the two warmest treatments, likely driving some of the negative effects of high temperature (Deane-Coe et al. 2015). Importantly, in field conditions, different species might experience different degrees of temperature-related drought depending on their habitat. Bryophytes growing in wet and aquatic ecosystems may experience less actual warming due to the temperature-buffering effects of water. Drier habitats, on the other hand, may experience surface temperatures much higher than 18 °C and, consequently, severe potential drought (Stoy et al. 2012). The actual effects of warming could therefore be both milder and more severe depending on species and their habitats.

Table 2. Average (±SE, n = 188) water-holding capacity of 10 bryophyte species expressed as % of dry biomass measured at the end of the experiment.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sphagnum fuscum</td>
<td>1857 ± 137²</td>
</tr>
<tr>
<td>Sphagnum compactum</td>
<td>1605 ± 74.7¹</td>
</tr>
<tr>
<td>Dicranum scoparium</td>
<td>825 ± 200bcd</td>
</tr>
<tr>
<td>Hylocomium splendens</td>
<td>799 ± 39.2b</td>
</tr>
<tr>
<td>Scorpidium revolvens</td>
<td>781 ± 61bc</td>
</tr>
<tr>
<td>Ptilidium ciliare</td>
<td>738 ± 148bcd</td>
</tr>
<tr>
<td>Pleurozium schreberi</td>
<td>725 ± 47.6bc</td>
</tr>
<tr>
<td>Barbilophozia floerkei</td>
<td>636 ± 70.2b</td>
</tr>
<tr>
<td>Racomitrium lanuginosum</td>
<td>534 ± 16.2cd</td>
</tr>
<tr>
<td>Racomitrium fusciculare</td>
<td>495 ± 29.2d</td>
</tr>
</tbody>
</table>

Notes: No temperature treatment is included. Different letters mark significantly different means (Tukey’s test).
Fig. 2. Average (±SE, n = 4) colony height growth or shoot length increment of 10 bryophyte species growing at five temperatures after 9 weeks. Colony height growth of *Sphagnum fuscum*, *Sphagnum compactum*, *Dicranum scoparium*, and *Barbilophozia floerkei* was measured with the cranked wires method (blue box around species name), and shoot length increment of *H. splendens*, *Scorpidium revolvens*, *Ptilidium ciliare*, *Pleurozium schreberi*, *R. lanuginosum* and *R. fasciculare* at individual shoots (red box around species name). Different letters mark significantly different groups (Tukey’s test). Species are ordered according to decreasing water-holding capacity (see SI 2).

**Fig. 2.** Average colony height growth or shoot length increment of 10 bryophyte species growing at five temperatures after 9 weeks. Colony height growth of *Sphagnum fuscum*, *Sphagnum compactum*, *Dicranum scoparium*, and *Barbilophozia floerkei* was measured with the cranked wires method (blue box around species name), and shoot length increment of *H. splendens*, *Scorpidium revolvens*, *Ptilidium ciliare*, *Pleurozium schreberi*, *R. lanuginosum* and *R. fasciculare* at individual shoots (red box around species name). Different letters mark significantly different groups (Tukey’s test). Species are ordered according to decreasing water-holding capacity (see SI 2).

**Important role in local moisture conditions at low than at high temperatures (Basilier et al. 1978).**

While nitrogenase activity was similar across species at 3 °C, bryophytes species varied greatly in their response to increasing temperature. Nitrogenase activity in *H. splendens* and *Pleurozium schreberi* peaked at 13 °C and then dramatically decreased at 18 °C demonstrating a clear optimum for N2-fixation. A similar pattern was recorded by Gundale et al. (2012a), where the intermediate, and not the greatest, warming treatment resulted in the highest nitrogenase activity in *H. splendens* and *Pleurozium schreberi*. This peak in nitrogenase activity in our study became more pronounced after 12 than 5 weeks, suggesting that the bryophyte-associated bacteria acclimated gradually to the temperature setting, as observed in earlier studies (Rousk et al. 2017). After 12 weeks, bacterial abundance or community structure may have changed in response to the persistent change in temperature (Warshan et al. 2016; Carrell et al. 2019). Both feather moss species had elevated tissue δ15N after 12 weeks, suggesting that the bacteria fixed more atmospheric N2 than they did in the tundra ecosystem where they were collected. Alternatively, such a change in δ15N could indicate a change of N form accessed through deposition as the experiment was highly controlled and no external N sources were available. Further, it is possible that the nitrogenase form in our study was eventually dominated by molybdenum-nitrogenase, which fixes nitrogen more efficiently at higher temperatures, compared to vanadium- and iron-nitrogenases of lower temperature optima (Miller and Eady 1988). *Hylocomium splendens* and *Pleurozium schreberi* naturally cover a large climatic range and are important components in the Arctic tundra and boreal forest understory (De Long et al. 2016; Bjerke et al. 2017). Depending on the specific climatic context, warming may strongly promote N2-fixation in *H. splendens* and *Pleurozium schreberi* in colder areas, while increasing temperatures in warmer boreal conditions could decrease fixation (Gundale et al. 2012a). Considering the high abundance of these species in some ecosystems, changes in associated N2-fixation could play a significant role in the N balance in the ecosystem.

In some bryophyte species, namely the semiaquatic *Scorpidium revolvens* and *Sphagnum* species, nitrogenase activity peaked at the warmest treatment. The higher temperature optimum of nitrogenase in these species was more pronounced after 5 than 12 weeks of temperature treatment suggesting direct effects of warming were important for the higher rates than long-term adaptations in bacterial communities. On shoots of *Scorpidium revolvens*, we observed 1 mm large spherical colonies of cyanobacteria. Free-living cyanobacterial colonies can sustain N2-fixation even when only partly hydrated (Kvíderová et al. 2011). Furthermore,
Fig. 3. (a) Average ($\pm$SE, $n = 4$) nitrogenase activity of 10 bryophyte species in five temperature treatments after (a) 5 and (b) 12 weeks of temperature treatment. Different letters mark significantly different means (Tukey’s test). Species are ordered according to decreasing water-holding capacity.
N$_2$-fixating bacteria are found inside special hyaline cells in *Sphagnum* spp. (Bragina et al. 2012), which may provide some protection from drying. Despite these water stress adaptations, the drop in tissue $\delta^{13}$C in the warmest treatment suggests that all bryophyte species and their microbial community were somewhat water limited in the warmest treatments after 12 weeks. It is possible that extended drought conditions negatively affected bacterial communities in *Scorpidium revolvens* and *Sphagnum* spp. and that nitrogenase activity in these species could have been promoted even more at 18 °C had the water not been limiting.

Nitrogenase activity in the remaining species was relatively low. Warming seemed to promote nitrogenase activity in *R. fasciculare*, *Dicranum scoparium*, and *Barbilophozia floerkei* whereas in *R. lanuginosum* and *Ptilidium ciliare*, the activity was unresponsive to warming. For *R. lanuginosum*, low activity is surprising, as substantial N$_2$-fixation has been measured in this species in other tundra ecosystems (Henriksson et al. 1987). We found no indication in the $\delta^{15}$N or $\delta^{13}$C data that *R. lanuginosum* performed differently than in the field, and it is therefore not clear why the activity was so low. Although liverworts are associated with N$_2$-fixing bacteria (Adams and Duggan 2008; Adams 2005), secondary compounds produced by some liverworts may prevent N$_2$-fixation (Gavazov et al. 2010), potentially explaining the low nitrogenase activity recorded for *Ptilidium ciliare*. The other liverwort, *Barbilophozia floerkei*, performed poorly in the experiment, and we are hesitant to speculate on the temperature responses in this species. Altogether, we found that 10 common tundra bryophyte species not only vary greatly in their bacteria-associated nitrogenase activity (Gavazov et al. 2010; Stuart et al. 2020), but also that they respond differently to warming. That bacterial acclimation to new climatic conditions depends on the bryophyte host identity is revealed by a delayed response of nitrogenase activity to increased temperatures, which was recorded only in the two feather mosses.

**Links between N$_2$-fixation, N concentration, and growth**

We expected warming to promote growth, partly through stimulation of N$_2$-fixation, which would increase N supply (Armitage et al. 2012; Berg et al. 2013). In line with our second hypothesis, nitrogenase activity and growth were overall positively correlated (SI 9). Growth of *Sphagnum compactum* and *Scorpidium revolvens* was high, and these species had a pronounced decline in shoot tip N concentrations, which suggests that growth was N limited as N$_2$-fixation still could not meet this demand. In our experiment, bryophytes could only access new N via atmospheric N$_2$-fixation and may have been N limited with the exclusion of N from deposition and throughfall which they would receive in natural settings (Zechmeister et al. 2007). However, *H. splendens* and *Pleurozium schreberi* had high nitrogenase activity and growth at 13 °C.
Fig. 5. Average (±SE, n = 4) shoot tip $\delta^{15}$N (‰) of ten different bryophyte species in five temperature treatments after 11 weeks relative to natural field condition concentrations (horizontal line, ±SE, dashed line, n = 4). Different letters mark significantly different groups (Tukey’s test). ∧ symbol marks temperature treatments significantly different compared to natural (field) conditions (Dunnett’s test). Species are ordered according to declining water-holding capacity.

and no concurrent decline in shoot N concentration, which suggests that N$_2$-fixation did compensate for the increased N demand in these species. Further, Pleurozium schreberi, H. splendens, and Dicranum scoparium had $\delta^{15}$N closer to 0 ‰, which additionally suggested that the contribution from atmospheric N$_2$ input increased during the experiment compared to the habitat where they were collected (Deane-Coe 2015). H. splendens and Pleurozium schreberi can actively attract and induce cyanobacterial colonisation (Bay et al. 2013), and they may have adapted to warming by stimulating the cyanobacterial community. That bacterial N$_2$-fixation contributed to shoot tip N concentration was further supported by a positive correlation trend between nitrogenase activity and N concentration across all species. However, based on the lack of significant relationship between $\delta^{15}$N and nitrogenase activity, we advise against using the isotopic N signature as a straightforward proxy for bacterial N$_2$ fixation in future studies.

Bryophyte growth and N acquisition responses will ultimately influence the role of bryophytes for C sequestration in the ecosystem. Bryophyte tissue generally decomposes slowly due to low N concentration (Lang et al. 2009; Turetsky et al. 2012), and the observed decrease in tissue N concentration coupled with enhanced bryophyte growth for some species at a higher temperature could further decrease decomposition rates (Gerdol et al. 2007; Britton et al. 2018). On the other hand, increased N inputs through deposition and warming-induced faster turnover of organic matter will increase N availability and favour vascular plants (e.g., graminoids and shrubs) (Elmendorf et al. 2012; Lett et al. 2017; Scharn et al. 2021). Existing research into how interactions between vascular plants and bryophytes may change with future climate change provides contrasting answers. Although emerging vascular vegetation in some ecosystems will pose a threat to various bryophyte species in a warmer climate (Elmendorf et al. 2012), a few studies find that Sphagnum and feather mosses successfully compete with shrubs under imposed warming (Dorrepaa et al. 2004; Keuper et al. 2011; Lang et al. 2012). Our study provides some suggestions for how this success may be linked to N dynamics.

Estimating landscape N$_2$ input

Applying two commonly used conversion factors of ethylene produced to N$_2$ fixed of 2.1 (Rousk and Michelsen 2017) and 3 (DeLuca et al. 2002), we can estimate the species-specific amount of N fixed at 9 °C, to represent fixation rates at temperatures closest to the summer average (SMHI) and at 13 °C, as a warming scenario in this region (IPCC 2018). If we combine these species-specific rates with the bryophyte species cover data from our two sites (Fig. 1), we get an estimated range of the total contribution of species examined...
in this study to the landscape N$_2$-fixation of $0.011 \pm 0.001$ to $0.016 \pm 0.002$ kg N ha$^{-1}$ month$^{-1}$ in Katterjåkk and $0.009 \pm 0.000$ to $0.013 \pm 0.001$ kg N ha$^{-1}$ month$^{-1}$ in Vassijaure. N$_2$-fixation in the warming scenario at 13 $^\circ$C, increases these values more than fivefold to ranges of $0.061 \pm 0.004$ to $0.088 \pm 0.006$ and $0.050 \pm 0.002$ to $0.072 \pm 0.003$ kg N ha$^{-1}$ month$^{-1}$ in the two sites, respectively. There are some uncertainties attached to these estimates relating to the conversion factors used and scaling from climate chambers. Inputs from bryophytes growing at generally moist conditions in the growth chambers might be overestimated for drier ecosystems. However, such conditions correctly characterise wet upland tundra ecosystems, such as these studied here, with bryophytes dominating the vegetation. Further, the acetylene reduction assay method may underestimate the fixation rates in Sphagnum due to different diazotrophic communities (Saiz et al. 2019). Nevertheless, the calculated warming-induced increases in N inputs through N$_2$ fixation correspond to plot-based field warming experiments (Gundale et al. 2012; Lett and Michelsen 2014). Importantly, this is one of the first attempts to estimate the warming response of bryophyte-associated N$_2$-fixation at the landscape scale, with a diverse bryophyte community. Ultimately, N inputs through increased N$_2$-fixation may lead to stimulation of moss growth, although responses may differ strongly between moss species, as demonstrated in our study.

**Conclusions**

We found large differences and nonlinearity of temperature responses in growth and N dynamics across the 10 bryophyte species, which correspond with large species differences in warming responses in field studies (Lang et al. 2012; Alatalo et al. 2020; Zuijlen et al. 2021). Warming particularly increased the growth of Sphagnum species, but their higher demand for N was likely not fully met by the increased N$_2$-fixation. Intermediate warming also increased the growth of the two circumarctic-boreal feather moss species, and this response seemed to link to an increased input of N via atmospheric N$_2$-fixation. Intermediate warming also increased the growth of the two circumarctic-boreal feather moss species, and this response seemed to link to an increased input of N via atmospheric N$_2$-fixation. This increased input of atmospheric N and C is further amplified by the high cover of feather mosses in sub- and low Arctic tundra. Our findings suggest that N$_2$ fixation and water-holding capacity, respectively, may be crucial for the success of boreal feather mosses and Sphagnum species at higher temperatures (Dorrepaal et al. 2004; Lang et al. 2012). The generally positive to neutral growth and nitrogenase activity responses across all the tested species suggests that warming per se is beneficial for bryophytes if water
is sufficient. However, species-specific growth responses may determine the outcome of competition with vascular plants in a future warmer world.

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Data availability
The data that support the findings of this study are openly available on figshare at http://doi.org/10.6084/m9.figshare.899105.

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Author contributions
AM and SL conceived the ideas and approach. AMR, AM, and SL designed the experimental setup. AMR and MANO collected the data. AMR analysed the data, and AMR and SL led the writing of the paper. All authors contributed critically to the drafts and gave final approval for publication.

Competing interests
The authors have no conflicts of interest to declare that are relevant to the content of this article.

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