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REGULAR ARTICLE

The resilience of nitrogen fixation in feather moss (Pleurozium schreberi)-cyanobacteria associations after a drying and rewetting cycle

Kathrin Rousk · Davey L. Jones · Thomas H. DeLuca

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Abstract
Background and aims Nitrogen (N_2) fixation in feather moss-cyanobacteria associations is a major source of N for boreal ecosystems. However, mosses experience significant shifts in their moisture status due to daily and yearly fluctuations in sunlight, temperature and precipitation. While the effects of drying and rewetting on nutrient leaching and photosynthesis in mosses have been studied, no attempt has been made to assess the consequences for N_2 fixation in feather mosses.
Methods We conducted an experiment in which we dried (3 day at 28 °C; <9 % of field moisture) and rewetted samples of the feather moss *Pleurozium schreberi* (Brid.) Mitt. that is colonized by N_2-fixing-cyanobacteria to assess the influence on N_2 fixation. Further, we tested how long it takes for N_2 fixation to recover from a drying-rewetting cycle. In addition, we assessed how N_2 fixation changes with incubation time with acetylene (2–65 h).
Results A drying period of 3 days almost completely eliminated N_2 fixation (<0.2 μmol m^{-2} h^{-1}) in the moss. However, rates slowly recovered after rewetting, reaching N_2 fixation levels of moist (non-water stressed) moss 5 days after rewetting. Nitrogen fixation increased significantly with incubation time with acetylene (0 μmol m^{-2} h^{-1} at 2 h vs. 26 μmol m^{-2} h^{-1} at 65 h incubation).
Conclusions Although N_2 fixation recommenced upon rewetting, the recovery was slow. Thus, recurrent drying and rewetting cycles could reduce total N_2 fixation in moss-cyanobacteria associations over time, leading to reduced total N input to the system.

Keywords Acetylene reduction · Cyanobacteria · Drought · Feather mosses · Nitrogenase · N_2 fixation

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K. Rousk · D. L. Jones · T. H. DeLuca
School of Environment, Natural Resources & Geography, Bangor University, Deiniol Road, Bangor, Gwynedd LL57 2UW, UK

K. Rousk (✉)
Section of Microbial Ecology, Department of Biology, Lund University, Lund, Sweden
e-mail: kathrin.rousk@gmx.net

T. H. DeLuca
School of Environment and Forest Sciences, University of Washington, Box 352100, Seattle, WA, USA

Introduction

Nitrogen (N_2) fixation performed by feather moss-cyanobacteria associations contributes about 2 kg N ha^{-1} $year^{-1}$ to N-limited boreal forests (DeLuca et al. 2002a; Gundale et al. 2011). Various abiotic factors influence the amount of N_2 that is fixed. For instance,

moisture (Gundale et al. 2009; Stewart et al. 2011), low N availability (Zackrisson et al. 2004; DeLuca et al. 2008), and higher rainfall frequency (Jackson et al. 2011; Gundale et al. 2012) have been found to positively affect N_2 fixation in cyanobacteria colonizing feather mosses and other bryophytes. Numerous studies suggest that moisture is the key driver of many processes in moss-cyanobacteria associations, including N_2 fixation (e.g. Smith 1984; Gundale et al. 2012). However, in the boreal forest, feather moss carpets can become extremely dry during long summer days and then rewet during subsequent heavy rainfall events, thus, experiencing natural and intensive drying and rewetting events. It has been shown that nutrient leaching and nutrient release from moss carpets after drying and rewetting is high (Carleton and Read 1991; Wilson and Coxson 1999). And while studies have addressed the effects of moisture additions to feather mosses and other bryophytes on N_2 fixation (Gundale et al. 2009, 2012; Jackson et al. 2011; Stewart et al. 2011), they have not addressed the effects of rewetting of dried moss.

Mosses can withstand drying until no free water remains in the cells and they quickly return to normal metabolism and growth upon rewetting. Additionally, the recovery of important biological processes (e.g. photosynthesis) can be rapid after rehydration in desiccation tolerant mosses, with some species starting to fix CO_2 immediately after rehydration. However, recovery time is highly dependent on species and habitat (Proctor 2001; Proctor et al. 2007).

One of the most abundant feather mosses in boreal forests is *Pleurozium schreberi* (Brid.) Mitt., which accounts for 60–90 % of the ground cover in mature boreal forests (Oechel and Van Cleve 1986; DeLuca et al. 2002b). *P. schreberi* is colonized by several species of cyanobacteria (*Nostoc* spp, *Stigonema* spp, *Calothrix* spp, *Cylindrospermum* spp.) (Gentili et al. 2005; Ininbergs et al. 2011), fixing substantial amounts of atmospheric N_2 (DeLuca et al. 2002a; Zackrisson et al. 2004). *Pleurozium schreberi* is poikilohydric (Glime 2007) and can tolerate desiccation due to its ability to enact cellular repair (Glime 2007). However, dry mosses are physiologically inactive and it can take up to 24 h after rehydration before full repair of damaged membranes occurs (Oliver and Bewley 1984). Thus, frequent and short sequences of desiccation of several hours up to 1 day seem to be more damaging than long periods of desiccation given the time required for full recovery (Oliver and Bewley 1984).

Cyanobacterial nitrogenase catalyzes the reduction of atmospheric N_2 in the *P. schreberi*—cyanobacteria association (Ininbergs et al. 2011; Leppänen et al. 2013). It has been shown that the recovery of nitrogenase activity in free-living and crust-forming cyanobacteria after desiccation is slower than the recovery of photosynthesis after desiccation due to de novo protein synthesis required for N_2 fixation (4 h vs. >24 h for photosynthesis and N_2 fixation, respectively) (Hawes et al. 1992; Belnap 2001). However, the rate of recovery of N_2 fixation in desiccated feather mosses after rewetting is currently unknown.

N_2 fixation is commonly measured with the acetylene reduction assay (ARA) (Schöllhorn and Burris 1967). This assay is based on the reduction of acetylene (C_2H_2) to ethylene (C_2H_4) performed by the cyanobacterial nitrogenase enzyme (Dilworth 1966). The production of ethylene in moss samples is usually measured after 24 h of incubation with acetylene, assuming a linear increase of N_2 fixation with incubation time (Zackrisson et al. 2004). Although acetylene reduction (AR) in the moss-cyanobacteria association is linear with time through 24 h (see Fig. S1, 2 h–24 h: r= 0.51; p=0.04; Gentili et al. 2005), there are no published reports assessing the behavior of AR in longer incubations. We propose that the AR assay is not linear in long-term incubations (greater than 24 h) due to nitrogenase-inhibiting levels of O_2 and CO_2 developing (Staal et al. 2001) as well as of inorganic N accumulating in the incubation vials. Therefore, incubation times in excess of 24 h could bias the results from the AR assay, which could lead to under- or overestimation of ecosystem N_2 fixation and with that, ecosystem N budgets. To assess the relationship between AR and incubation time, we incubated samples of *P. schreberi* with acetylene and measured AR at various time points after incubation over a 65 h period.

We then used our knowledge of the linearity of AR to examine the influence of extreme drying and rewetting events on N_2 fixation in feather mosses and to estimate the amount of time needed for the cyanobacterial nitrogenase enzyme to recover from desiccation and to recommence fixing N_2. With this set of studies, we specifically aimed to answer the following questions: (1) How quickly and to what degree do *P. schreberi*-cyanobacteria associations reach N_2 fixation rates that are comparable to rates in constant moist moss after rewetting of air-dried moss? and (2) How does N_2 fixation in *P. schreberi* change with incubation time with

acetylene? These collective findings fill an important gap in the literature and may account for some of the spatial and temporal variability previously observed in the field (DeLuca et al. 2002a; Zackrisson et al. 2004). Further, the results generated in this set of studies could have implications for estimations of ecosystem-N-fluxes using N_2 fixation data obtained with the AR technique as well as regarding extreme drying conditions.

Materials and methods

Drying-rewetting and acetylene reduction time series—sampling and experiments

In August 2011, moss shoots of *P. schreberi* were collected at a late succession forest in northern Sweden (299 years since last fire; Zackrisson et al. 1996). The sampling site is located at 65.4375°N, 18.4440°E. Mean annual temperature and precipitation are 1 °C and 570 mm, respectively. The vegetation was dominated by Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L. (Karst)), feather moss carpets composed of *P. schreberi* and *Hylocomium splendens* (Hedw.), *Vaccinium vitis-idea* (L.), *V. myrtillus* (L.) and *Empetrum hermaphroditum* (Hagerup). The site has been described previously (Ackermann et al. 2012) and moss samples from this site have been shown to fix sufficient amounts of N_2 to be used in our experiments (Ackermann et al. 2012). From the time of sampling until 1 week before the start of the experiments, the moss was kept in a cold room (5 months at 5 °C) to slow down any activity. One week before the start of the experiments, moss samples were put into a growth chamber at 10 °C, 16 h light (500 μmol m^{-2} s^{-1})/8 h dark cycles to re-activate moss and cyanobacteria.

For the acetylene reduction time series experiment, as well as for the drying-rewetting experiment, three replicate samples (each sample consists of 10 moss shoots) for each measuring time point were used. The moss shoots were placed in 20 ml glass vials and kept at room temperature (~20 °C) and ambient lighting over the course of the experiments. Due to the required incubation with acetylene prior to the ethylene measurements, the samples were destructively sampled for each measurement. A total of 48 samples (each consisting of 10 moss shoots) were used for the drying-rewetting experiment, from which half were kept moist (24 constantly moist samples) and half were dried and rewet (24 samples). Acetylene reduction was measured at 8 different time points after rewetting, with three replicates for each measurement (total $n=8$ time points × 3 replicates × 2 treatments=48). In the AR-time series experiment, we measured AR at 8 different time points with 3 replicates each (total $n=24$): 2, 4, 8 17, 24, 40, 48 and 65 h after incubation with acetylene.

For the drying and rewetting experiment, half of the glass vials with moss shoots (24 vials) were used as control samples and kept moist over the course of the experiment (constantly moist moss). The second half (24 vials) was subjected to desiccation by air-drying: the vials were placed in a warm greenhouse (approximately 28 °C) for 72 h un-lidded and with constant airflow induced by a fan. This temperature reflects surface moss temperatures observed in the summer months at the field site. The control samples were placed in the same greenhouse but kept moist by leaving the vials lidded. Moisture in these samples was maintained with distilled water as necessary. Acetylene reduction was measured in all samples at the start of the experiment, when all samples were still moist (24 h incubation time). Wet weight of moss samples was determined prior to drying. When the desiccated moss was below 9 % moisture, AR was measured again in the dried and constantly moist samples. The dried moss was rewetted with distilled water to the starting wet-weight and AR was measured 2, 4, 8, 16, 24, 32, 48 and 120 h after rewetting in the dried-rewet and in the constantly moist moss. After all measurements, moss samples were dried at 80 °C for 3 days and weighed.

N_2 fixation analyses

N_2 fixation analyses were performed using the ARA (Schöllhorn and Burris 1967) as described by Zackrisson et al. (2004). Briefly, 20 ml glass vials containing 10 moss shoots were sealed and 10 % of the headspace was replaced with acetylene. Moss samples were incubated for 24 h at room temperature (~20 °C) in the drying-rewetting experiment. Various incubation times were used in the acetylene reduction time series experiment (see above). Ethylene generated in the headspace by the cyanobacterial nitrogenase enzyme was measured by gas chromatography equipped with a flame ionization detector (Varian, Santa Clara, USA) using an automatic headspace sampler (Quma, Wuppertal, Germany). Data are represented as cumulative AR in

nmol g^{-1} DW, as total AR in μmol m^{-2} or as AR rates in μmol m^{-2} h^{-1} where 10 moss shoots of *P. schreberi* represent an area of 2.8 cm^2 (Rousk et al. 2013).

Statistical analyses

Differences in AR between the dried moss and the constantly moist moss upon rewetting of the dried moss was analysed with Analysis of Variance (ANOVA) approaches followed by Tukey's Post Hoc Test. Linear regression analyses were used to analyse the relationship between AR and time after rewetting of the dried-rewet moss. Welch-T-Tests were used to test for differences in AR before and after drying as well as to test for differences in moss weight between the treatments (dried-rewet vs. constantly moist moss). To test for differences in AR between the incubation times with acetylene in the time series experiment, ANOVAs were used followed by Tukey's Post Hoc Test. The relationship between AR and incubation time with acetylene was analyzed with linear regression analysis. Analyses were performed using R 2.14 (R Development Core Team 2011). The cumulative AR was best described with a power fit equation calculated in KaleidaGraph 4.1.3. (Synergy Software).

Results

Acetylene reduction time series experiment

Total AR as well as AR rates increased significantly with incubation time (r=0.84; p<0.001; r=0.80; p<0.001 for total and rates, respectively) with an exponential increase after 40 h of incubation with acetylene (Fig. S1). While hardly any AR was measured at 2 h (0.9±0.5 μmol m^{-2} h^{-1}; mean±SE), mean AR rates increased to 23.4±1.1 μmol m^{-2} h^{-1} by 65 h of incubation with acetylene (F=38.2; p<0.001).

Drying and rewetting experiment

At the time of measurement, the same amount of biomass and percent moisture was present in mosses for the two treatments (dried-rewet moss and continuously moist moss) as shown by the comparable dry and wet weights (Table 1). There was also no significant difference in AR between the mosses before the drying: mean AR rates in the moss before drying were 2.5±0.04 (SE) μmol m^{-2} h^{-1} and in the constantly moist moss 2.7±0.06 μmol m^{-2} h^{-1} (t=−1.4; p=0.18). After drying, AR was significantly reduced in the dried moss compared to AR in the same moss before drying (0.15±0.007 μmol m^{-2} h^{-1}; t=−9.9; p<0.001), but remained similar in the continuously moist moss (4.0±0.2 μmol m^{-2} h^{-1}) and thus, was significantly different in the two treatments before rewetting (t=−4.8; p<0.001) (Table S2).

After rewetting of the dried moss, clear differences between the constantly moist moss and the dried-rewet moss were observed (Figs. 1 and 2). The continuously moist moss showed more than 10-fold higher AR rates as well as cumulative AR levels measured at the same time intervals throughout the experiment than the dried and rewetted moss (F=21.2; p<0.001). Acetylene reduction in both treatments increased significantly until 48 h after rewetting (r=0.72; p=0.0001, r=0.58; p=0.003 for the dried-rewet and continuously moist moss, respectively) (Figs. 1 and 2). But after 48 h, AR in the continuously moist moss did not increase further in contrast to the dried-rewet moss (Figs. 1 and 2). Rates of AR were positively correlated with time after rewetting in the dried-rewet moss (r=0.41; p=0.03) and reached levels comparable to the constantly moist moss 5 days after rewetting (Fig. 2). However, AR in the dried-rewet moss remained low over the course of the experiment, with a mean of 139±112 nmol g^{-1} DW and a maximum cumulative value of 238 nmol g^{-1} DW 120 h after rewetting, which was four times lower than in the continuously moist moss (940 nmol g^{-1} DW). Interestingly, mean AR rates in the continuously moist moss were more than ten-fold higher after 48 h (6.9±0.5 μmol m^{-2} h^{-1}) than after 120 h (0.67±0.9 μmol m^{-2} h^{-1}) (Fig. 2).

Discussion

Acetylene reduction time series

Acetylene reduction in the moss increased with incubation time with acetylene wherein incubation times of 40 h or longer resulted in exponentially higher N_2 fixation rates than shorter incubation times. Thus, incubation times of samples that are compared in regard to their N_2 fixation rates should be as similar as possible and ideally less than 40 h. Alternatively, N_2 fixation could be re-calculated incorporating the specific incubation times assuming a linear relationship of N_2 fixation rates with

Table 1 Wet and dry weight (g) of 10 shoots of *Pleurozium schreberi* used in the drying-rewetting experiment. Given are mean values ± SE. Wet weight for all moss samples was determined before the experiment (100 % moisture), oven-dry weight was determined after the experiment (80 °C, 3 days). Also given is the mean ± SE for the air-dried moss (~28 °C, 72 h in the green house) as well as moisture content (%) on a fresh weight basis

	Wet weight (g)	Oven-dried (g)	Air-dried (g)
Constantly moist moss	3.12±0.14	0.16±0.01	–
Dried-rewet moss	3.12±0.09 (100 % moisture)	0.14±0.005	0.27±0.04 (8.6 % moisture)

time for incubations less than 40 h only. Generally, long incubation times (>40 h) should be avoided since the samples are exposed to conditions that could inhibit nitrogenase activity (e.g. increased levels of CO_2, O_2) (Staal et al. 2001), making extended incubations increasingly unrealistic for estimating N_2 fixation under natural conditions (Hardy et al. 1973). However, it should be emphasized that the ARA remains a highly sensitive and efficient means of estimating N_2 fixation (Hardy et al. 1973; Leppänen et al. 2013).

Drying and rewetting

Our study provides the first look at the influence of desiccation on N_2 fixation in *P. schreberi* and demonstrates that recovery upon rewetting is slow. It took 5 days for N_2 fixation in the dried-rewet moss to reach levels comparable to levels in the continuously moist moss. Fast, as well as short drying and rewetting cycles have been found to be more damaging to moss than slow drying and longer cycles since protective and repair mechanisms have to occur (Proctor et al. 2007). The contrary has been found for cyanobacteria: the longer the cells are drought-stressed, the longer it takes to recover nitrogenase activity (Scherer et al. 1984; Belnap 2001). It should be emphasized that in a field setting, moss moisture contents would be influenced by microclimatic conditions and their intimate connection to a humus mass capable of holding a great deal of moisture.

Although recovery of N_2 fixation in the dried and rewet moss was slow, it did recommence after rewetting, suggesting that 3 day air-drying at 28 °C is sufficient enough to reduce N_2 fixation almost to zero and at the same time not to completely destroy all cyanobacteria cells and their activity. It is likely that the cyanobacteria entered a dormant state during the drying of the moss. Upon improvement of the conditions (rewetting), the cells revived and resumed their activity (Kaplan-Levy et al. 2010). Gundale et al. (2012) also found N_2 fixation in *P. schreberi* recovered from increased temperatures (+2 °C)

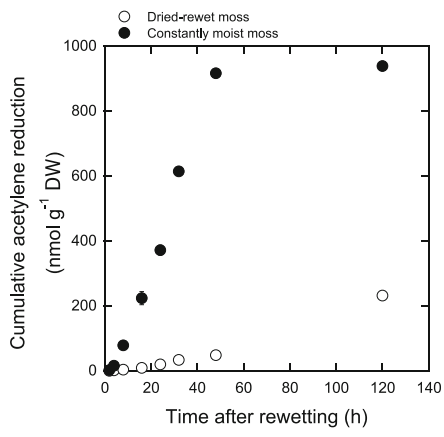

Fig. 1 Cumulative N_2 fixation (nmol acetylene reduced g^{-1} DW) in *Pleurozium schreberi* in the dried-rewet moss (*open symbols*) and in the constantly moist moss (*filled symbols*) at different time points after rewetting (2–120 h). Shown are means ± SE. Error bars are sometimes hidden behind symbols

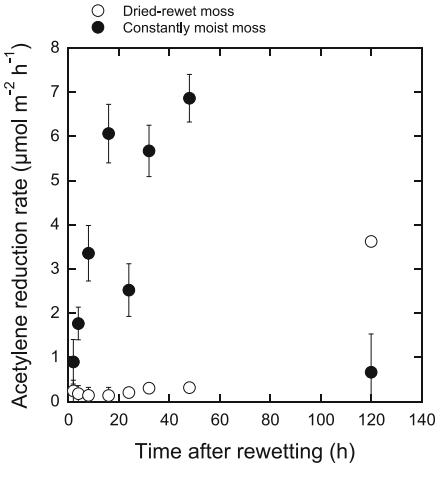

Fig. 2 Mean acetylene reduction rates ± SE (µmol m^{-2} h^{-1}) in the dried-rewet moss (*open symbols*) and in the constantly moist moss (*filled symbols*) in relation to time after rewetting. Error bars are sometimes hidden behind symbols

and less frequent rainfall events in the field. However, recovery was slow (weeks up to months).

The drop in N_2 fixation in the continuously moist moss at the last sampling point could be the result of O_2 and CO_2 accumulating in the vials, impairing N_2 fixation rates (Smith 1984; Staal et al. 2001). However, lids were taken off regularly to adjust for water loss and to allow for gas exchange. The sharp decline in N_2 fixation could also indicate a down-regulation of nitrogenase activity as a result of N accumulation in moss leaves following several days of high N_2 fixation activity and without a desiccation period. Nitrogenase activity is end product inhibited and since the constantly moist mosses were kept in the same vials from the start of the experiment, an accumulation of N in moss leaves could have led to an inhibition of the enzyme (Hardy et al. 1973).

Recovery time of fundamental biological processes like photosynthesis, respiration and N_2 fixation is highly dependent on the duration of the desiccation period as well as on species, habitat and pre-collection condition (Belnap 2001; Proctor et al. 2007). However, N_2 fixation seems to recover slower than photosynthesis and respiration after rewetting. This could be the result of de novo synthesis of proteins required for N_2 fixation (Hawes et al. 1992; Belnap 2001), re-activation of dormant cyanobacterial cells (Kaplan-Levy et al. 2010), recolonization and the need for differentiation of heterocysts where N_2 fixation occurs (Scherer et al. 1984; Belnap 2001). Further, N_2 fixation is an extremely energy demanding process, thus energy must first be generated via photosynthesis to be able to regain nitrogenase activity (Belnap 2001; Turetsky 2003; Houlton et al. 2008). This could lead to a lag phase before N_2 fixation can recommence after drying and rewetting of mosses. Belnap (2001) proposed such a lag-phase in N_2 fixation in cyanobacterial soil crusts in which respiration and photosynthesis almost immediately recommenced after rewetting. This N_2 fixation lag-time is dependent on the carbon (C)-stores, the nitrogenase level and the numbers of heterocysts, which are in turn fundamentally influenced by past and current environmental conditions that drive photosynthesis (Belnap 2001).

Scherer and Zhong (1991) found decreasing N_2 fixation rates after drying in free-living cyanobacteria, but stability increased after several drying cycles, suggesting that the cyanobacteria developed a desiccation tolerance induced by repeated cycles of rewetting and desiccation (Scherer and Zhong 1991). Moss-cyanobacteria-associations in boreal forests could be adapted to drying and rewetting cycles since they experience large variations in their hydration status over the course of only few hours (Startsev and Lieffers 2002). Further, feather mosses and the associated fibric organic horizon are known for their ability to retain water (Turetsky 2003) and could represent moist spots in the boreal understory in contrast to other plants, facilitating colonization by cyanobacteria.

When measuring the effects of desiccation on N_2 fixation rates in free-living cyanobacteria, Scherer and Zhong (1991) found no nitrogenase activity below 71 % water content of fully rewetted colonies. Although not directly comparable to N_2 fixation in cyanobacteria, mosses can stay active (respiration, photosynthesis) at water contents as low as 20 % (Proctor et al. 2007). Thus, free-living cyanobacteria seem to require higher moisture conditions for their activity than mosses and moss-associated cyanobacteria. Even though the moisture content of the air-dried moss was very low (8.6 %, see Table 1), we could detect low activity of the cyanobacterial nitrogenase enzyme, suggesting that the moss is able to maintain a suitable environment for the cyanobacterial colonizer, offering a more favorable habitat than the free-living state.

In our experiment, recovery of N_2 fixation was reached 5 days after rewetting, whereas complete recovery of photosynthesis is reported to be reached only few hours after rewetting in mosses (Proctor et al. 2007) and in cyanobacteria (Hawes et al. 1992; Satoh et al. 2002; Belnap 2001). However, we have to keep in mind that the feather moss-cyanobacteria association is an alliance of two different organisms, thus, a distinction between enzymatic processes in the plant versus that in bacteria is difficult. The feather moss-cyanobacteria association is assumed to be mutualistic (Turetsky 2003), in which both partners exchange N and C with each other. But if both partners are exposed to stressors like drying and rewetting, moss, as well as cyanobacteria, have to invest energy in membrane repair and photosynthesis (and N_2 fixation) (Glime 2007; Turetsky 2003). Thus, the question emerges which partner recovers faster to be able to provide and exchange resources with the partner. Mosses are active at high relative humidity (Kidron et al. 2002; Herrnstadt and Kidron 2005) while cyanobacteria require liquid water for their activity (Lange et al. 1986, 1998); and photosynthesis seems to recover faster than N_2 fixation after rewetting (Hawes et al. 1992; Belnap 2001; Proctor et al. 2007). These observations suggest that mosses could start fixing C

earlier than cyanobacteria start fixing N_2 after rewetting, and could provide the cyanobacterial partner with energy and C, promoting the recovery of nitrogenase activity, which ensures an endogenous N supply for the moss. This is an interesting, but understudied area of research that calls for further studies. One way forward would be to expose moss-cyanobacteria associations to recurrent drying-rewetting cycles and to measure N_2 fixation as well as photosynthesis over the course of several hours. Mosses with varying numbers of cyanobacterial colonizers should be used in order to assess if the cyanobacteria remain photosynthetic active when colonizing the moss.

However, as stressed by Belnap (2001), the recovery of N_2 fixation is highly dependent on the species composition, biomass, habitat etc. We assessed the effects of drying-rewetting on N_2 fixation in one moss species colonized by several genera of cyanobacteria. This could make it difficult to draw conclusions for other species and ecosystems. Nevertheless, *P. schreberi* is a common, and often dominant feature in many ecosystems. A recent study indicates further that the host-moss is a more sensitive indicator for the associated cyanobacterial community than environmental factors (Ininbergs et al. 2011). At least four different genera have been identified on *P. schreberi* leaves, which could have different and complementary tolerance levels towards environmental stressors (see e.g. Gentili et al. 2005). These collective findings imply that we can draw general conclusions about the effects of moisture-stress on N_2 fixation in the *P. schreberi*-cyanobacteria association, and not only for the boreal forest.

Light is an important driver for photosynthesis and N_2 fixation. However, when living in symbiosis or in a loose association, the importance of this factor could likely become negligible. Given that mosses are able to photosynthesize at a wide range of light intensities, as well as under dry conditions, the cyanobacteria likely receive C from the moss, excluding light as the limiting factor for nitrogenase activity in these associations. The exchange of N and C as well as the different activity optima of the partners (moss and cyanobacteria) in regard to moisture could explain the success of *P. schreberi* in occupying various habitats around the world.

Our results suggest that dry periods during summer could lead to a significant reduction of N_2 fixation in the moss-cyanobacteria association. Furthermore, changes in temperature and precipitation patterns, as predicted for the future, will fundamentally affect N_2 fixation in moss-cyanobacteria associations. Most likely, N_2 fixation will be highly impaired as a result of desiccated moss, resulting in less N input to the system through this pathway. On the other hand, we showed that N_2 fixation is capable of recovery from desiccation stress upon rewetting and further, mosses are known to release large amounts of nutrients upon rewetting (Carleton and Read 1991; Wilson and Coxson 1999). Thus, feather-moss cyanobacteria associations could represent a significant N source immediately upon rewetting via moss leachates and a longer-term N source via continuation of N_2 fixation by the cyanobacterial associates. However, when the moss is optimally hydrated to promote N_2 fixation, it is likely that the fixed N_2 is incorporated into moss tissue (Bay et al. 2013), which is supported by the known efficiency of mosses to retain and sequester nutrients (e.g. Oechel and Van Cleve 1986).

Our study is the first attempt to assess the effects of drying-rewetting events on N_2 fixation in moss-cyanobacteria associations. In addition, it has raised numerous questions to be addressed in future studies and highlighted potential difficulties for assessing N_2 fixation on an ecosystem scale. To overcome the problems associated with spatial and temporal variability of moisture when sampling mosses, researchers should use many replicates and consider pre-sampling conditions. Furthermore, in order to assess the impacts of natural drying-rewetting cycles on N_2 fixation in boreal forests, the degree of the drying event needs to be carefully evaluated to assess the actual degree of desiccation. Ideally, N_2 fixation should be assessed in parallel with photosynthesis rates and cyanobacterial colonization during these fluctuations in hydration status in the feather moss-cyanobacteria association.

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