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Water availability controls nitrogen fixation in the feather moss, *Pleurozium schreberi*

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Abstract The influence of water availability in terms of amount and frequency of addition on N fixation of the feather moss *Pleurozium schreberi* was evaluated in a greenhouse experiment. Moss was collected in the Vaksliden forest reserve, northern Sweden, and subjected to several watering treatments. Three volumes of estimated monthly rainfall, low (20 L/ m^2month), medium (60 L/m^2month) and high (100 L/m^2month) where each applied at three different frequencies, infrequently (every 15 days), medium frequency (every 5 days) and frequently (every day). As a general trend, N fixation was reduced when low volumes of water were applied and increased with medium and high water volumes. The increase in N fixation was related not only to the amount of water, but how frequently this water was added. As such, N fixation decreased as the interval between consecutive watering events was increased. The effect of the watering regime in the release of inorganic N (NO_3^--N, NH_4^+-N) and P (PO_4^{3-}-P) through the moss layer was measured by installing resin capsules under the moss layer. Overall, these nutrients were released in higher amounts when medium and high volumes of water were added except in the case of NO_3^--N. NO_3^--N which is highly soluble was found in higher amounts than any other nutrient, favored by the addition of low volumes of water. The release of NH_4^+-N was higher when the moss layer was subjected to infrequent watering. Although water availability was found to be an important factor controlling N fixation in *P. schreberi*, its role in the leakage of N as a source to the ecosystem remains to be investigated.

Keywords *Pleurozium schreberi*, N fixation, moisture, nutrient release

Introduction

Feathermosses and their association with cyanobacteria are one of the main agents of N input in boreal ecosystems (De Luca *et al*., 2002; Houle *et al*., 2006). Nitrogen has often been considered the main limiting nutrient for primary production and other important forest ecosystem processes (Vitousek and Howart, 1991; Vitousek *et al*., 2002), especially in high latitude ecosystems. The feathermoss *Pleurozium schreberi* (Brid) Mitt. and its associated cyanobacteria are amongst the few organisms able to provide N in cold regions, and cyanobacterial fixation activity has shown to play a very important role in high latitudes (Stewart, 1969; Alexander and Schell, 1973; Alexander and Billington, 1986; Chapin *et al*., 1991; Potts, 1994). The moss layer houses a large amount of nutrients in boreal forest soils and it is a very important component of the forest biomass in boreal forests (Bonan and Shugart, 1989). In addition, bryophytes, being poikilohydric organisms, also act as passive sinks for nutrients in all ecosystems, yet a high portion of all the nutrient inputs come as atmospheric deposition or through rainfall (Mäkipää, 1995; Turetsky, 2003). Feathermosses are widespread along the whole boreal range, but also Asia, South America and Africa (De Luca *et al*., 2002; Houle *et al*., 2006).

Even though the physiology of biological N fixation is well understood, the ecological controls that drive cyanobacerial fixation are still unclear (Vitousek *et al*., 2002). Ecological factors that are characteristic of late secondary successional boreal forests appear to increase the rate of fixation, (Zackrisson *et al*., 2004; De Luca *et al*., 2007; Lagerström *et al*., 2007;), but which factors are responsible and how they are related to the dynamics of cyanobacteria are still poorly understood. Time since last fire and available N are both strongly correlated with N fixation (Zackrisson *et al*., 2004). Other variables such as temperature, light intensity, and moisture have also been shown to be correlated with fixation (Alexander and Schell, 1973; Dickson, 2000; Gentili *et al*., 2005; Zielke *et al*., 2002; Zielke *et al*., 2005). As terrestrial cyanobacteria are photosynthetically active organisms, water availability appears to be among the most important factors influencing their metabolism (Potts, 1994). Accordingly, their nitrogenase activity (as measured by the acetylene reduction technique (Schöllhorn and Burris, 1967)) has been shown to be affected by the availabilty of water in cyanobacterial colonies (Rodgers, 1977; Potts and Bowman, 1985). Other studies have focused on how dessication in bryophytes affects important metabolic processes such as photosynthesis, that may regulate N fixation by the cyanobacteria that they host (Skre and Oechel, 1981; Skre and Oechel, 1983; Proctor, 2001; Proctor *et al*., 2007).

However, how precipitation influences N fixation in the feathermoss-cyanobacteria association has not yet been adressed. Rainfall may have an effect on N inputs and outputs from feathermoss carpets. The foreseen increase in precipitation and temperature in northern Europe due to climate change will definitely affect forest ecosystems in many ways and undoubtedly to nutrient fluxes (IPCC, 2007). Hence, further research is needed to better understand the inputs and ouputs in the N cycle.

Moreover, managed boreal forests, especially in the northernmost Scandinavian range have been fertilized to compensate for their low N availability (Demoling *et al*., 2008). However, changes in soil N content may have negative ecological consequences. First, N fixation by feathermosses is directly reduced after the application of N fertilizers (DeLuca *et al*., 2007). Following fertilization, microbial biomass and activity are reduced and abundance of ground vegetation such as feathermosses or lichens are also often reduced (Olsson and Kellner, 2006; Demoling *et al*., 2008). A better understanding of the factors regulating N fixation should lead to an improvement in the application of fertilizers in Fennoscandian and boreal forestry, being relevant to forest management.

However, even if N is fixed in the widespread feathermoss carpet in the boreal range, the availability of this nutrient to co-existing plants or other organisms in boreal ecosystems is still poorly understood and further research is needed (Lagerström *et al*., 2007). It has been suggested that N contained in mosses may be released and mineralized during the slow decomposition of the moss layer (Alexander and Billington 1986). Nevertheless, some studies have pointed how water passing through the moss layer, after desiccation episodes, could promote the short-term release of nutrients (Weber and Van Cleve, 1983; Startsev and Lieffers, 2006).

This study reports a greenhouse experiment aimed at investigating how artificial rainfall events applied at different quantities and frequencies influence N fixation in the feathermoss *P. schreberi*. It is hypothesized that a deficiency in water availability will negatively affect the metabolism of the *P. schreberi*-cyanobacteria association, thus affecting N fixation rates. Moreover, this study investigates whether the quantity of water, the frequency in watering, and interactive effects between these two variables affect N fixation rates.

In addition, this work seeks to investigate if artificial rainfall influences the release of nutrients from the moss layer. After periods without water, a sudden water addition may lead to a release of nutrients from the damaged tissues. This study will therefore allow determination of whether water amount, watering frequency or an interactive effect of the two is responsible of this release. This will help us to understand the role of water in the short-term release of nutrients from the moss layer and if this is significant for the ecosystem.

In a more general sense, this work will also aim to assess how rainfall events may influence nutrient ecosystem dynamics in a changing climate and allow us to better understand the functioning of nitrogen pools in boreal forests.

Materials and methods

Biological material

Bryophyte samples were collected at Vaksliden forest reserve (65º42´N, 18º45´E) in northern Sweden during September 2007. The stand consisted of a late successional *Pinus sylvestris* L. dominated forest (297 years since last fire) of ericaceous-cladina type on sandy glacial soils classified as either Typic or Entic Haplocryods (Berglund *et al*., 2004). The moss *P. schreberi* dominated the forest-floor layer and ericaceous shrubs *Empetrum hermaphroditum* Hagerup and *Vaccinium myrtillus* L. are the most abundant understorey shrub species. The general soil characteristics of the site are: 4.14 pH, 44.98% Humus C; 1.00% Humus N; 1.2% soil C and 0.04% N (Berglund *et al*., 2004).

A total of 50 microplots, each 300 x 300 mm were sampled at the study site. The microplots were selected at sites where *P. schreberi* was clearly dominant, and away from the direct shadow of trees or shrubs. The humus layer was removed from the microplots to avoid the presence of tree roots or decomposed matter. All microplots were placed in plastic square boxes of the same size and stored in a greenhouse at SLU, Umeå until February 2008. The microplots were kept moist by watering them daily with 10 L of distilled water until the start of the experiment. Distilled water was used to avoid any contribution of nutrients to the moss. All shoots of *P. schreberi* was thoroughly cleaned of litter, impurities and other plant species.

144 black plastic plots measuring 70 x 70 x 70 mm were filled with 15 mm of substrate, a mixture of 60% sand (Baskarpsand), 30% peat (Weibulls Trädgård) and 10% of organic matter from the study site. One filter paper (Munktell filterNo.3) was added at the bottom of the pot to avoid soil losses. Then, moss from all microplots was homogenized and approximately 40g of moist moss was placed into each of half (72) of the pots. The weight of moss

Table 1. Watering volumes and frequencies for the different treatments and total volume added to each treatment before the N fixation measurements

Volume of water	Frequency in watering	No. of treatment occasions	Monthly volume (L/m^2)	Treatment duration before N fixation measurement	Total volume added in L/treatment (16 pots)
Low	Each 15 days	1	20	25 days	0.80
	Each 5 days	5			1.33
	Daily	25			1.32
Medium	Each 15 days	1	60		2.40
	Each 5 days	4			3.20
	Daily	25			4.00
High	Each 15 days	1	100		4.00
	Each 5 days	4			5.31
	Daily	25			6.60
Low	Each 15 days	3	20	51 days	2.40
	Each 5 days	10			2.66
	Daily	51			2.69
Medium	Each 15 days	3	60		7.20
	Each 5 days	10			8.00
	Daily	51			8.16
High	Each 15 days	3	100		12.00
	Each 5 days	9			11.95
	Daily	51			13.46

added to each pot was determined experimentally by weighing samples with an area of 4900 mm^2 (area of a pot) in the original microplots.

Experimental design and watering regime

Pots with and without moss were randomly paired off and assigned to one of a total of nine watering regimes, 8 replicates were assigned on each watering regime with a total of 72 pairs and 144 pots. The watering regimes were based on precipitation data from the study site with an average monthly precipitation of 57 L/m^2 for the summer season, and a period without rain as long as 15 days (SMHI, 2008). Three levels for monthly watering were defined: Low quantity (L) = 20 L/m^2; medium quantity (M) = 60 L/m^2 and high quantity (H) = 100 L/m^2. Three levels for frequency in rainfall events were determined: Infrequently (I) = each 15 days; medium frequency (N) = each 5 days and frequently (F) = every day (Table 1). The volume of water added to each treatment was adjusted in accordance with the total surface of the pots per treatment in L/m^2. All the conditions in the greenhouse were controlled during the experiment to assure that N fixation was only affected by the watering treatment. The greenhouse conditions were set to mimic those inside a boreal forest during the summer season. Hence, the temperature was 18ºC during daytime and 12ºC at night with a 18 hours daytime period, air humidity in the room was maintained between 50-60% and light intensity was 120 µmoles photons s^{-1} m^{-2} , measured with a Decagon sunfleck ceptometer (Delta-T devices LTD). The watering regime was applied for 51 days from February 17 to April 9, 2008.

Nitrogen fixation analysis

Nitrogen fixation rates were estimated using a calibrated acetylene reduction technique (Schöllhorn and Burris, 1967). Measurements were taken once prior to the start of the experiment and following 25 and 51 days after the start of the experiment. Ten moss shoots were randomly removed from each pot and placed into a 22-ml glass culture tube. The tubes were each fitted with a septum and 10% of the total headspace was evacuated and replaced with acetylene. The tubes were incubated for 24 h under greenhouse conditions before analysis for total ethylene production on a Perkin Elmer, Clarus 500 GC and Turbomatrix 40 headspace injector. The samples were injected isothermically onto a 50 m Perkin Elmer Elite-Alumina capillary column (0.053 mm ID). Ethylene standards were used (0, 57, 570 ppm) to estimate sample ethylene concentrations. *Pleurozium schreberi* shoots were oven dried for 48 hours at 65ºC. Afterwards, estimates of ethylene production rates per unit moss weight (nmol g^{-1} day^{-1}) were performed using the universal gas law (PV=nRT). The data are thus presented as the amount of acetylene reduced, which is closely correlated with

N fixation. DeLuca *et al.* (2002) described that the *Pleurozium-Nostoc* association and other *Pleurozium-cyanobacteria* associations reduce acetylene at a ratio of 3 mol of ethylene per mol of N fixed.

Nutrient availability

To asses available inorganic N (NH_4^+-N, NO_3^- -N) and P (PO_4^{3-}-P), one resin capsule (UNIBEST PST-1 Resin Capsule) was installed on top of the soil in each pot by slightly digging with a rounded tube and placing it in the resulting hole. In the case of pots containing moss, the capsule was placed at the interface between the moss layer and the soil surface (De Luca *et al*., 2007). In each control (moss-free) pot only half of the capsule was buried, so that the upper part remained visible. Resin extracts were analyzed for available inorganic N and P 51 days after the start of the experiment. Capsules were placed in centrifuge tubes and three sequential aliquots of 10 mL of 2 M KCl desorption solution were added. Centrifuge tubes where shaken for 30 minutes after the addition of each aliquot. A total 30 mL eluate was obtained from each capsule and stored in the refrigerator at 5ºC until further analysis (De Luca *et al*., 2007). Resin capsule eluates were analyzed for PO_4^{3-} using the molybdate–ascorbic acid method (Kuo, 1996), NO_3^-–N by using the cadmium reduction method and NH_4^+–N using the salicylate–nitroprusside method (Mulvaney, 1996) by using an Autoanalyzer III (SEAL Analytical, Omni Process, Stockholm.). Results were expressed as mg/L of nutrient from the 30 mL resin capsule extract.

Statistical analysis

The experiment was set up as a full factorial design (3 water volume treatments x 3 water frequency treatments) and was therefore analyzed using a two-way ANOVA. The dependent variable was obtained by substracting the acetylene reduction measure prior to the start of the experiment from the acetylene reduction measures 25 days and 51 days after the start of the experiment. Data were tested to determine whether they met with the assumptions of ANOVA; Kolmogorov-Smirnov and Shapiro-Wilk were used to test normality while Levene’s test was used to test for homogeneity of variances. Data were log-transformed to satisfy these assumptions when required. Significant differences at $P \leq 0.05$ between treatment means were determined with Tukey’s post hoc test and Student-Newman-Keuls test. The release of nutrients due to mosses was estimated as the difference between the concentrations found in pots with moss and pots without moss, and this release was also analyzed by two way ANOVA, (on log-transformed data), as described above. All data were analyzed with SPSS 15.0 for windows (SPSS, Chicago, Ill).

Results

Effects of the watering regime on N fixation

Nitrogen fixation showed a general increase over time after implementation of the watering regime for all the treatments except for those treated with low volumes of water in which N fixation decreased (Figure 1). The different watering volumes already started to influence N fixation 25 days after the start of the experiment. At this time, low volumes of water led to a statistically significant decrease in N fixation, whereas medium and high volumes of water increased N fixation rates (Figure 1a). After 51 days the addition of low volumes of water was responsible for the greatest decrease in N fixation. These rates of N fixation differed significantly from those in the medium and high water volume treatments which still showed the highest increase (Figure 1b).

The different frequencies did not have any important effect on N fixation after 25 days. However, the interaction between the volume of water and the frequency in watering had a significant effect on N fixation after 51 days.

An interactive effect between the volume of water and the frequency of watering was found at the end of the experiment (Table 2). The effect of low and high water volumes on N fixation depended strongly on how frequently these volumes were applied, whereas with medium volumes of water the effects of watering frequency were less important.

Time also found to have an effect on N fixation. It appears that some period is needed until the treatments start to have an effect on N fixation. Moreover, differences in N fixation rates between the treatments became larger with increasing duration of the experiment was maintained in time.

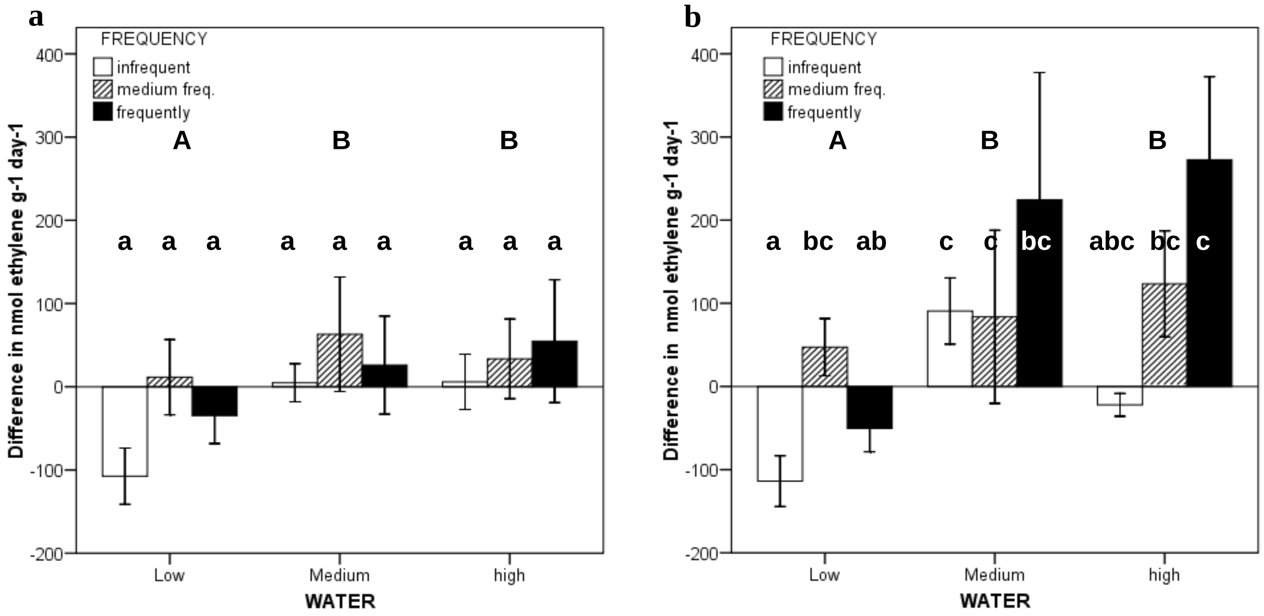

Figure 1. Difference since the start of the experiment in N fixation rates (as estimated by acetylene reduction) after 25 days (**a**) and 51 days (**b**). Bars represent the mean acetylene reduction value of 8 replicates per treatment. Units expressed as nmol ethylene g^{-1} dry moss day^{-1}. Error bars represent +/-SE Capital letters indicate significant differences between water treatment means (Tukey's test, $P \leq 0.05$), and small letters indicate significant differences between frequency treatment means for each volume treatment (Tukey's test, $P \leq 0.05$).

Table 2. Analysis of variance of the difference in N fixation rates (as estimated by acetylene reduction) in nmol ethylene g^{-1} dry moss day^{-1}, using water and frequency as factors, 25 and 51 days after the start of the experiment.

Dependent variable	Source	Type III sum of squares	Degrees of freedom	Mean square	F Value	P value
nmol ethylene g^{-1} day^{-1} (after 25 days) [1]	WATER	2.749	2	1.375	4.406	0.016
	FREQUENCY	1.339	2	0.669	2.146	0.125
	W * F	1.927	4	0.482	1.544	0.200
	Error	19.655	63	0.312		
nmol ethylene g^{-1} day^{-1} (after 51 days) [1]	WATER	7.271	2	3.636	10.889	<0.001
	FREQUENCY	1.994	2	0.997	2.986	0.058
	W * F	3.429	4	0.857	2.568	0.047
	Error	21.035	63	0.334		

[1] Using log transformed values.

Effects of the watering regime in the release of nutrients

Amounts of inorganic N (NO_3^--N, NH_4^+-N) and P(PO_4^{3-}-P) released by the moss (calculated as the difference between pots with moss and pots without moss), were significantly influenced by the watering regime. In general, for all the nutrients analyzed, the addition of medium and high volumes of water resulted in release from *P.schreberi*. The addition of low volumes of water had different effects on the release of the different nutrients (Figure 2). When low volumes of water where added, NO_3^--N was strongly released by *P.schreberi*. However, the same volume of water resulted in an uptake of NH_4^+-N or PO_4^{3-} -P (Figure 2).

Interactive effects between the volumes of water and the watering frequencies were found for NO_3^--N and PO_4^{3-}-P at the end of the experiment (Table 3). Therefore, the effects of the watering frequencies differed for different water volumes when the release of NO_3^--N and PO_4^{3-}-P but not NH_4^+-N was considered. One important effect of the watering regime was the high concentration of NO_3^--N present when a low volume of water and a medium watering frequency was applied, relative to the other treatment combinations (Figure 2a). This value was significantly different (*P* <0.001) from those of all other treatments.

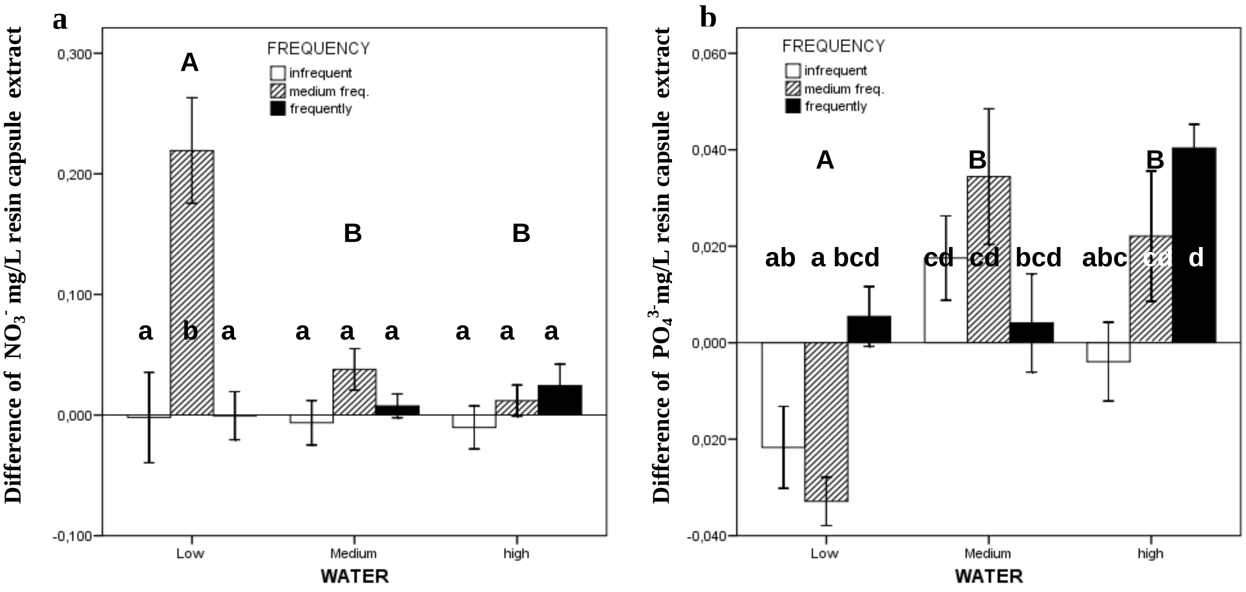

Figure 2. Amounts of NO_3^- -N (**a**), PO_4^{3-} -P (**b**) and NH_4^+-N (**c**), expressed as the difference in concentrations found between pots with *Pleurozium schreberi* and pots without *P. schreberi*. Units are mg/l of nutrient per resin capsule extract (30 mL). Bars represent the mean concentration per treatment (n=8). Error bars represent +/-SE. Capital letters indicate significant differences between water treatment means, and small letters indicate significant differences between frequency treatment means for each volume treatment (Tukey's test, P ≤ 0.05).

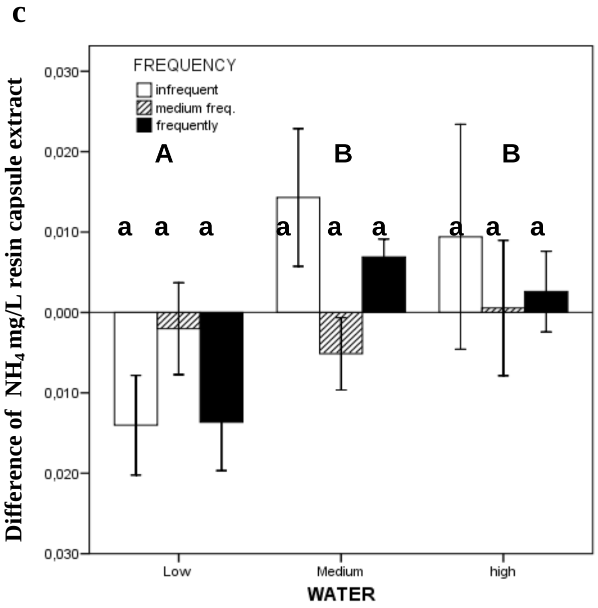

Table 3. Analysis of variance for the amounts of NO_3^--N, PO_4^{3-}-P and NH_4^+-N released by *P.schreberi* in mg/L per resin capsule, using water and frequency as factors, 60 days after the start of the experiment.

Dependent variable	Source	Type III sum of squares	Degrees of freedom	Mean Square	F Value	P value.
NO_3^--N (mg/L) [1]	WATER	0.007	2	0.004	5.563	0.006
	FREQUENCY	0.018	2	0.009	13.383	<0.001
	W * F	0.020	4	0.005	7.457	<0.001
	Error	0.041	63	0.001		
PO_4^{3-}-P (mg/L) [1]	WATER	0.003	2	0.002	14.924	<0.001
	FREQUENCY	0.001	2	0.000	3.348	0.042
	W * F	0.002	4	0.001	4.868	0.002
	Error	0.007	63	0.000		
NH_4^+-N (mg/L)	WATER	0.003	2	0.002	3.911	0.025
	FREQUENCY	0.000	2	0.000	0.465	0.630
	W * F	0.002	4	0.001	1.254	0.298
	Error	0.028	63	0.000		

[1] Data log transformed.

Overall, *P. schreberi* released more NO_3^--N than any other nutrient at the end of the experiment, whereas the lowest concentrations detected were found for NH_4^+-N (Figure 3).

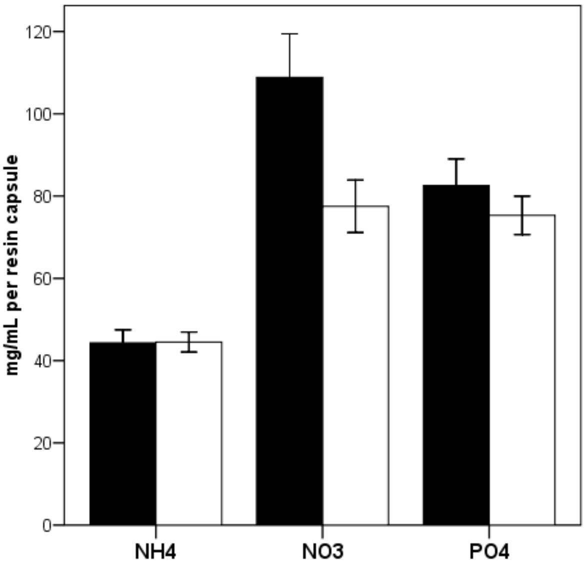

Figure 3. Amounts of NO_3^--N, PO_4^{3-}-P and NH_4^+-N found in pots with *Pleurozium schreberi* (black bars) and without *P. schreberi* (white bars). Units are mg/L of nutrient per resin capsule extract (30 mL). Bars represent the mean concentration found in all of the replicates across treatments n=72. Error bars represent +/-SE.

Discussion

The results of this experiment showed that N fixation by cyanobacteria living in association with *Pleurozium schreberi* was limited by the availability of water, both in terms of quantity and frequency. Although many authors have recognized the importance of water content and desiccation periods in regulating the nitrogenase activity of cyanobacteria (Rodgers 1977, Scherer and Zhong, 1991), the decrease in N fixation observed due to water limitation may affect basic metabolic processes in cyanobacteria hosted by *P. schreberi*.

Water limitation negatively affected N fixation by cyanobacteria, consistent with earlier studies showing optimal N fixation at high water saturation levels (Rodgers, 1977; Scherer and Zhong, 1991). When cyanobacteria are rewetted, nitrogenase activity is activated following enhancement of respiration and photosynthesis (Scherer et al., 1984). If the quantity of water is not enough to activate these processes, then N fixation may be limited. In addition, low water contents may be sufficient to allow some recovery of nitrogenase activity of current cyanobacterial colonies, but not high enough for the germination and development of new individuals (Rodgers, 1977).

Overall, the decrease in N fixation in the low water treatments became stronger with increasing duration of the desiccation period. This is in accordance with several previous studies in which the recovery in nitrogenase was found to be rapid after short drought periods but to take progressively longer as the drought period was extended (Kershaw and Dzikowski, 1977; Scherer *et al*., 1984 Potts and Bowman, 1985).

In this sense, a constant moist condition should keep moss metabolic processes and growth of cyanobacteria in optimal conditions, except when the water level is too low or moss shoots are affected by long desiccation periods. Moreover, dry-wet cycles should also lead to reduced rates of N fixation. Although, most of the effects can be explained in terms of water and frequency combinations, some of the variability observed in N fixation rates, must be the result of a natural variability of cyanobacteria or heterocysts present in the moss shoots (Scherer *et al*., 1984; DeLuca *et al*. 2007).

Another possible aspect that should be further investigated to understand the mechanisms of N fixation is the relationship in the cyanobacteria-*P.schreberi* association. Cyanobacteria may reduce their photosynthetic rates and become dependent on the C made available by the host to accomplish N fixation (Rai *et al*., 2000). It is possible that the C available for cyanobacteria was reduced in *P. schreberi* by the influence of low water volumes as well. Therefore, desiccation would reduce moss photosynthesis rates (Skre and Oechel, 1981) and rewetting of moss desiccated tissues would contribute to a release of C as carbohydrate leachates (Turetsky 2003) or as CO_2 through respiration as damaged tissues are repaired (Startsev and Lieffers, 2007).

Less strong, but still significant were the effects of the watering regime on the release of N (NO_3^--N, NH_4^+-N) and P (PO_4^{3-}-P). In general, each of these nutrients was released from the moss layer when medium or high volumes of water were applied. In natural conditions, water passing through moss after rainfall has been shown to increase release of mineral N (Alexander and Schell, 1973; Weber and Van Cleve, 1983). Under laboratory conditions, soaking of moss has also shown to cause a release of N and P through leaching (Carleton and Read, 1990; Startsev and Lieffers, 2006). In this study, when low volumes of water were added, only NO_3^--N showed a very high release as a result of *P.schreberi* being present, whereas for NH_4^+-N and PO_4^{3-}-P no release due to *P.schreberi* was observed. As desiccation should be stronger in moss shoots treated with low volumes of water, this should lead to a higher release of nutrients due to moss tissue damage (Carleton and Read, 1990; Starstev and Lieffers, 2006), or at least the same amount of nutrients that we found in pots without moss shoots. A possible explanation for this would be that low water volumes passing through the moss layer might not be sufficient to dissolve and thus release nutrients from them. Another explanation for the low amount of nutrients found could involve the process of capillarity when *P. schreberi* is moist, leading to movement of nutrients from the soil to the moss layer (Starstev and Lieffers, 2006).

The watering regime had opposite effects on the release of the main N forms, NO_3^--N and NH_4^+-N. NO_3^--N, being a more soluble form, was generally found in higher amounts as long as sufficient water volumes were added. The release of NH_4^+-N was not so strongly related to water addition. It could be that tissue damage after long desiccation periods resulted in higher amounts of NH_4^+-N being produced by the moss. This would help protect contents of the cells from leaking out, thus converting N into less soluble forms (Starstev and Lieffers, 2006).

In this experiment, however, it was only possible to quantify the concentrations of nutrients found in the resin capsules rather than identify their source. Therefore, it was difficult to address how much of these nutrients were released from *P. schreberi* tissues, soil in the pots sorbed by the resin capsules by capillarity or fixed by cyanobacteria.

In boreal forests as well as in other high latitude ecosytems, water appears to be a prevailing factor controlling N fixation in feathermosses (Alexander and Schell, 1973; Billington and Alexander, 1983; Chapin *et al*., 1990). With the predicted increase in temperature and precipitation in high latitude ecosystems resulting from global climate change, it is very likely that N fixation in *P. schreberi* will be increased (IPCC, 2007). However, the availability of this fixed N for co-existing plants is still unknown and faster decomposition rates of dead moss due to global warming could lead to an increased release of mineralized N forms. On the other hand, precipitation is not likely to have a significant effect on a short-term release of nutrients from the moss layer. Although desiccated moss tissues may release nutrients when rewetted, rainfall events that may provide enough water to leach these soluble forms from moss tissues must be rare and infrequent in nature (Startsev and Lieffers, 2006), and therefore unlikely to be important in the ecosystem nutrient cycle.

This study shows how feathermoss is important in determining input of N to the ecosystem from atmospheric N_2 and how water is a major factor controlling this input. However, the extent to which N fixed by cyanobacteria in mosses can be released from the moss layer is still unresolved and the effect of water in a short-term release of nutrients from the moss layer needs further research.

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