



**The effect of moisture, litter, and stand age
on N fixation in the feathermoss,
*Pleurozium schreberi***

*Kvävefixering hos *Pleurozium schreberi* i sena och tidiga
brandsuccessioner: betydelse av fukt och förna*



Foto: Anders Jäderlund

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I denna rapport redovisas ett examensarbete utfört vid Institutionen för skogens ekologi och skötsel, Skogsvetenskapliga fakulteten, SLU. Arbetet har handledts och granskats av handledaren, och godkänts av examinator. För rapportens slutliga innehåll är dock författaren ensam ansvarig.

This report presents an MSc thesis at the Department of Forest Ecology and Management, Faculty of Forest Sciences, SLU. The work has been supervised and reviewed by the supervisor, and been approved by the examiner. However, the author is the sole responsible for the content.

ABSTRACT

The largest known natural input of nitrogen (N) into the boreal forests occurs through biological fixation by cyanobacteria that live in association with the moss *Pleurozium schreberi*. There is currently limited knowledge about the factors responsible for the variation in N fixation rates, and specifically what factors drive variability between stands. In this study I have studied the N fixation rate on *P. schreberi* in a watering experiment and a litter extract experiment. In the water experiment the effect of frequency of water additions were studied, while in the litter experiment the effects of litter extracts of the three most common tree species and three of the most common dwarf shrub species of the boreal forest were studied. The moss was collected from three young successional sites and three old successional sites after fire. The study was performed in a greenhouse for a period of 45 days and N fixation rates were estimated after 13, 21 and 45, 43 days for water experiment and litter experiment respectively using a calibrated acetylene reduction technique. I found that the mosses collected from young stands had significantly lower N fixation rates compared to mosses collected from old stands. Watering treatments had a significantly positive effect on N fixation rates in mosses originating from old, but not from young stands. The watering effect on the N fixation rate was increasing from measurement on the 13th day of treatment to day 45. For the litter extract experiment, no significant effect of any litter type was detected. These results suggest that micro variation at the stand level in water availability is likely a more important determinant of ecosystem N fixation rates in old successional forests than is the deposition of foliar compounds on moss carpets.

SAMMANFATTNING

Det största kända naturliga tillskottet av kväve (N) till boreala skogar sker genom biologisk kvävefixering hos cyanobakterier som lever i nära association med väggmossa (*Pleurozium schreberi*). Kunskapen om de ekologiska faktorer som påverkar cyanobakteriernas kvävefixering är begränsad och detta gäller i synnerhet de faktorer som kan förklara den stora variationen i kvävefixering mellan olika skogsbestånd. Syftet med detta arbete var att studera hur vattentillgång och olika förnaextrakt påverkar kvävefixeringen hos *P. schreberi*. Detta gjordes dels genom ett bevattningsexperiment där mossor vattnades med olika tidsintervall och dels genom ett förnaextraktexperiment där mossor vattnades med förnaextrakt framställt från förna hos de tre vanligaste träarterna och de tre vanligaste risväxterna i de studerade bestånden. Mossan som användes i försöken var insamlad från tre unga och tre gamla brandsuccessioner nära Arvidsjaur i Norrbottens län. Experimenten utfördes i ett växthus under en 45 dagars period. För vattenexperimentet beräknades kvävefixeringen (med hjälp av kalibrerad acetylenreduktionsteknik) efter 13 och 45 dagars behandling och för förnaextraktexperimentet efter 21 och 43 dagar. Resultaten visade en signifikant lägre kvävefixering hos de mossor som var insamlade i de yngre brandsuccessionerna jämfört med mossor som samlats in från de äldre successionerna. Vatten hade en generellt signifikant positiv effekt på kvävefixeringen hos mossor som var insamlade från äldre successioner, men inte hos mossor från yngre successioner. Effekten av vattenbehandlingen ökade från dag 13 till dag 45. Tillsats av förnaextrakt hade ingen signifikant effekt på kvävefixeringen. Resultaten från denna studie antyder att vattentillgången i äldre brandsuccessioner förmodligen har en större betydelse för att förklara den mikovariation i kvävefixering hos *P. schreberi* som finns i olika bestånd än vad läckage (eller urlakning) av ämnen från döda blad av olika arter har.

INTRODUCTION

Nitrogen (N) is considered to be the factor most limiting productivity in northern boreal forests (Tamm 1991). Following fire disturbance, young successional stands exhibit high availability of N (DeLuca *et al.* 2002b) relative to old successional stands, despite a much lower total soil N pool (Zackrisson *et al.* 2004). This high N availability corresponds with high stand productivity. As succession proceeds, N availability diminishes (DeLuca *et al.* 2002b), and new N enters the forest through biological fixation and atmospheric deposition (Zackrisson *et al.* 2004). The result is an increasing total soil N pool with successional-age, with a majority of N becoming increasingly occluded into highly recalcitrant and unavailable humic N. Biological N fixation, therefore, is profoundly important because it replenishes the forest soil with new N and provides a temporary un-occluded form of N that could contribute to plant productivity. The largest known natural input of N into the boreal forests occurs through biological fixation by cyanobacteria that live in association with the moss *Pleurozium schreberi* (Brid.) Mitt. (Schreber's big red stem moss) (DeLuca *et al.* 2002a). The bacteria live epiphytically on the leaves of the moss (Zackrisson *et al.* 2004), eventually getting access to carbohydrates and protection from the moss in return for N (Zielke *et al.* 2005).

There is currently limited knowledge about the factors responsible for the variation in N fixation rates, and specifically what factors drive variability between stands. It has previously been reported that the highest rate of N fixation occurs in old successional forests (Zackrisson *et al.* 2004), where the highest density of mosses and cyanobacteria are also found (DeLuca *et al.* 2007). It is generally known that excess of N in the environment reduces biological N fixation (Chapin *et al.* 1991), and fertilization experiments of *P. schreberi* have shown that enhanced levels of available N reduces N fixation of the associative bacteria (Zackrisson *et al.* 2004). Biological N fixation is also generally thought to be phosphorus (P) limited, although the results from terrestrial experiments with P additions show variable responses, e.g. as a stimulating effect (Israel 1987), neutral, very small effect (Zackrisson *et al.* 2004), or a strong negative effect (Smith 1984). However, there are several additional factors that may be important drivers of N fixation rates, such as the availability of water (Turtesky 2003) and the species composition of a stand. Experimental evaluation of the relationship between these additional factors and N fixation rates in boreal ecosystems are scarce.

Water availability may be a critically important regulator of N fixation in boreal stands because cyanobacteria, like all organisms require moist membranes for biological activity (Chapin *et al.* 1996), and because H^+ ions hydrolysed from water have a key role in reducing N_2 to NH_3 during N fixation (Stevenson and Cole 1999). The fact that *P. schreberi* is non-vascular, makes it highly susceptible to drying, and thus water may be an important regulator for the cyanobacteria-moss association. Likewise, forest species composition may be an important regulator of N fixation rates due to the influence of litter or throughfall inputs into the moss layer. Litter chemistry varies considerably between boreal species (Wardle *et al.* 2003), and several chemical attributes of litter, such as polyphenol or total N content, vary substantially between species (Kuiters and Sarink 1986) and have been shown to influence a variety of nitrogen cycling processes (Baldwin *et al.* 1983, White 1986, Schimel *et al.* 1998). Few studies have investigated the effects of plant litter on N fixation (but see Schimel *et al.* 1998). No research on feather mosses in boreal forests has yet evaluated the importance of water availability or

litter chemistry on N fixation rates, yet both of these factors are known to vary with successional age.

In my thesis I tested the following three hypotheses;

- (a) N fixation rate is positively correlated with water availability because water limitation reduces activity of cyanobacteria.
- (b) Plant litters have varying effects on N fixation rates because of their contrasting chemistries.
- (c) Water and plant litter treatments will have similar effects in stands of different successional ages because the same genera of bacteria are found on mosses independent of time since fire (DeLuca *et al.* 2007), and because the N fixation process could be assumed to respond similarly irrespectively of stand age.

By addressing these questions I aim to contribute to a better understanding of how moisture and a variety of litter types influence N fixation rates on *P. schreberi* in the boreal forest, and whether and how these effects vary in forest stands of different successional age.

MATERIAL AND METHODS

Site descriptions

The materials for this study were collected from six sites in the northern boreal zone of Sweden (65°34' - 65°56' N, 18°14' - 19°06' E) (Table 1). The sites were selected because they vary in different characteristics and because low influence of traffic and air pollutions. Three of the sites have recently burned (45-106 years since last fire) and three sites have had longer development since last fire (290-360 years) (Table 1). For all sites, the tree layer consists of *Pinus sylvestris* L. (Scots pine), *Picea abies* L. (Karst.) (Norway spruce) and some scattered *Betula pendula* Roth. (Silver Birch) and *B. pubescens* Ehrh. (Downy Birch) (Table 2). The ground vegetation in all six sites is dominated by ericaceous dwarf shrubs and feather mosses, mainly *Vaccinium myrtillus* L. (Bilberry) and *Pleurozium schreberi*, respectively. The young sites have a higher proportion of grass, mainly *Deschampsia flexuosa* (L.) Trin. (Wavy hairgrass), *V. myrtillus*, *V. vitis-idaea* L. (Lingonberry), and the tree layer is dominated by *P. sylvestris*. The older sites have a larger proportion of *Empetrum nigrum ssp. hermaphroditum* (Lange ex Hagerup) Böcher (Black crowberry) and *P. schreberi* in the forest floor, and the relative proportion of *P. abies* increases with stand age (Table 2). All mineral soils developed in granitic glacial till or glacial sediment and have a 4.3 to 6 cm thick Oe/Oa horizon (Table 3), 10 to 20 cm thick E horizon and a 30 to 40 cm thick Bs horizon (Zackrisson *et al.* 2004). Soils are classified as either Typic Haplocryods or Entic Haplocryods. For chemical characteristics of each stand see Table 3 and DeLuca *et al.* (2007).

Table 1. Location and year of burning for the six study sites. Data are from DeLuca *et al.* (2007) and Gundale (*unpublished*).

	Järvliden	Granliden	Avaviken	Vaksliden	Kuottavare	Ruttjeheden
Year of burning	1962	1924	1901	1716	1694	1647
Elevation (m)	514	489	499	434	532	467
Latitude (N)	65°34'	65°34'	65°36'	65°43'	65°56'	65°46'
Longitude (E)	18°24'	18°20'	18°36'	18°44'	18°14'	19°06'

Table 2. Percentage of vegetation cover (%) for dominant understory vegetation types and total basal area (m² ha⁻¹) for dominant tree species at the study sites. Data are from DeLuca *et al.* (2007) and Nilsson (*unpublished*).

	Järvliden	Granliden	Avaviken	Vaksliden	Kuottavare	Ruttjeheden
Trees (basal area m ² ha ⁻¹)						
<i>Pinus sylvestris</i>	12	19	19	-	5	16
<i>Picea abies</i>	3	1	5	-	10	16
<i>Betula pendula</i> / <i>B. pubescens</i>	0	0	2	-	2	0
Dwarf shrubs (% cover)						
<i>Vaccinium myrtillus</i>	15.8	37.5	35	-	27.5	23.3
<i>Vaccinium vitis-idaea</i>	20	9.2	12.5	-	7	7.5
<i>Empetrum hermaphroditum</i>	4.8	2	12.5	-	21.7	24.2
Other dwarf shrubs*	1	0	0	-	1	3
Feather mosses (% cover)						
<i>Pleurozium schreberi</i>	9.2	40	50.8	-	70.8	65.8
<i>Hylocomium splendens</i>	1	12.5	2.3	-	7.8	3
Grass (% cover)						
<i>Deschampsia flexuosa</i>	5.8	8.3	5.3	-	2.3	1

**Vaccinium uliginosum*, *Calluna vulgaris*.

- data not available

Field sampling

During 10-12 September 2007, light green- yellowish shoots of *P. schreberi* were collected from about 30 locations in each of the six sites. The collection of moss was done at least 100 m from any road to prevent influence of traffic. When possible, the collection was done in canopy gaps to prevent direct chemical influence from the tree layer and to reduce the variability among stands. After collection, the mosses were transported to the laboratory, and air dried at room temperature (22°C) for a week in order to remove any initial differences in moisture content.

Senescent leaves (litter) of the three major tree species and the three dominant dwarf-shrubs were collected from the site Vaksliden on September 12, 2007. The species collected were *P. sylvestris*, *P. abies*, *Betula sp.*, *V. myrtillus*, *V. vitis-idaea* and *E. hermaphroditum*. The collection of litter was done from stems and thin branches of each of the different plant species. The collection of litter from *E. hermaphroditum*, *V. myrtillus*, and *P. abies* was done by cutting off stems or thin branches where many dead leaves were attached. The branches were then brought inside and kept at room temperature, allowed to dry, and then the leaves were removed from the branches by shaking. All litter was air dried at 28°C for one week, and sorted from other types of organic material.

Experimental set-up

Mosses were returned to the laboratory and transplanted into plastic pots (0.01m²) for a litter extract experiment and a watering experiment, respectively. Both experiments utilized a similar experimental approach, where individual pots were established by placing a Munktel 3 filterpaper on the perforated bottom, followed by 150-180 g of rinsed quartz sand (Baskarpssand). The moss shoots were first cleaned from fresh litter and then placed on top of the sand. Each pot was filled with approximately 3.87-9.12 g (mean 6.61 g) (dry weight) of moss, equalling approximately 133-263 (mean 181) moss

Table 3. Chemical characteristics of humus and moisture content of *Pleurozium schreberi* for the three young (eg. Järvliden, Granliden, Avaviken) and the three old (eg. Vaksliden, Kuottavare, Ruttjeheden) forest study sites. Data is expressed as mean (\pm SE) values for. $n=3$ Data are from Gundale and Nilsson (*unpublished*).

	Young	Old
Total N content (g kg^{-1})	10.2 \pm 0.9	20.8 \pm 11.8
Total content (mg kg^{-1})		
P	774 \pm 96	690 \pm 73
Al	1357 \pm 172	939 \pm 151
Ca	2198 \pm 445	1946 \pm 102
K	856 \pm 123	801 \pm 97
Fe	13666 \pm 253	896 \pm 171
Mn	499 \pm 45	261 \pm 83
Mg	316 \pm 65	334 \pm 19
C:P	536 \pm 29	640 \pm 44
N:P	10 \pm 0.0	27 \pm 12
C:N	40 \pm 0.8	35 \pm 12
Ca:Al	2 \pm 0.4	2 \pm 0.6
Available nutrients (mg kg^{-1})		
NH_4^+	61 \pm 9	62 \pm 6.6
NO_3^-	6.5 \pm 3	4.0 \pm 1.4
P	217 \pm 36	192 \pm 34
Ca	205 \pm 38	143 \pm 5
K	518 \pm 97	475 \pm 58
Mn	66 \pm 9.4	29 \pm 8
Mg	65 \pm 15	57 \pm 4
Humus (Oi) depth (cm)	5.3 \pm 0.3	4.8 \pm 0.5*
Moisture of humus (%) ¹	62 \pm 5	76 \pm 1
Moisture of moss (%) ¹	78 \pm 2	81 \pm 2

* $n = 2$

¹Data collected during 10-12 September 2007.

shoots per pot. The difference in weight and number of shoots was due to different size of the moss shoots. The goal was to have the same density in each pot.

For each of the two experiments (see below) the moss pots originating from each site were randomly arranged in 5 blocks, which represented 5 sub-replicates of each treatment. All pots were kept in the greenhouse under artificial light with a 12 h day and 12 h night light regime, and with an average day and night temperature of approximately 20°C and 14°C, respectively. The light intensity at the top of the mosses during the day was 270-320 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (PAR). The mosses were re-wetted with de-ionized water to establish similar moisture conditions in all pots before the start of the experiments. The moss in the watering experiment was allowed to acclimatize in the greenhouse for 3 days before the start of treatment, while the moss of the litter extract experiment had 10 days to acclimatize.

All pots were randomly redistributed within the block during the experiment. The position of each block was also rotated every fourth day during the experiment to assure an equal distribution of background variation among all blocks and all treatments. All pots were placed in larger low boxes for practical purpose with free drainage. During treatment application, the pots were removed from these boxes to prevent unwanted water movement between different treatments. Prior to treatment applications a pre-treatment measurement of N fixation was made (see below) to compare the fixation rate between forest study sites.

Table 4. Number of water treatment occasions and the total amount of water added to each pot for each of the different water treatment at the time of N fixation measurement.

Treatment duration before N fixation measurement	Frequency of treatment	No. of treatment occasions	Total volume of H ₂ O added (ml 0.01 m ⁻² pot)
13 days	Daily	13	650
	Every second day	7	350
	Every third day	5	250
	Every fourth day	4	200
	No water	0	0
45 days	Daily	45	2250
	Every second day	23	1150
	Every third day*	16	800
	Every fourth day	12	600
	No water	0	0

*On one occasion, all 30 pots were watered one day too early

(a) Watering experiment

In order to test the influence of water availability on N fixation rates of *P. schreberi* originating from old and young successional forests, an experiment consisting of five watering treatments was set up. The water treatment consisted of five different levels of watering; (i) moss watered every day; (ii) moss watered every second day; (iii) moss watered every third day; (iv) moss watered every fourth day; and (v) moss without water added. At each watering occasion, each pot was amended with 50 ml of de-ionized water, which delivered enough water to allow some excess run-off. Water treatments were initiated on 24 of September, 2007. The first measurement of N fixation of moss was done 13 days later, and the second N fixation measurement was performed 45 days after experiment initiation. This watering regime resulted in 45, 23, 16, 12, and 0 water applications for each of the water treatments during the course of the experiment (Table 4). At time of sampling, moss used for N fixation measurements were wetted to similar moisture content.

(b) Litter extract experiment

To determine if different types of litter affect N fixation rates in *P. schreberi*, an experiment that consisted of watering with litter extracts from the three dominant tree species and the three dominant dwarf shrubs species (see Field sampling) was set up. I chose to use extracts of litter instead of adding the litter directly onto the moss. This was mainly done to avoid difference in light conditions. The moss pots were watered every second day and arranged in the green house as described above. Litter extracts were prepared for each litter type by sequentially extracting litter from each species. The extraction began by adding 0.85 g of litter per pot in 50 ml of de-ionized water. The extracts were shaken for 24 h on a rotator (90 rpm) at room temperature (22°C), followed by filtration through Munktel No 5 filter paper. Litters were oven dried at 28°C, and sequentially extracted every other day for 43 days (Table 5). To simulate the gradual input of fresh litter onto the forest floor, 0.12 g of fresh litter were added 15 times throughout the duration of the experiment. After each extraction, 50 ml of the extract was applied to each of the 5 replicate pots per site. My goal with this extraction protocol was to maintain the presence of any highly soluble compounds in the extract that may only be present in fresh litter, while also creating an accumulating pool of older litter, which may release more recalcitrant compounds through the repeated soaking

Table 5. Schedule of amount of litter used for preparing each litter extract. Data are expressed as g (dry weight) litter added per 50 ml water for each day of treatment.

Day	New fresh litter added (g)	Cumulative litter used (g)
1	0.85	0.85
3	0	0.85
5	0	0.85
7	0.12	0.97
9	0.12	1.09
11	0.12	1.21
13	0.12	1.34
15	0.12	1.46
17	0.12	1.58
19*	0.12	1.7
21**	0	1.7
23	0	1.7
25	0	1.7
27	0	1.7
29	0	1.7
31	0.12	1.82
33	0.12	1.94
35	0.12	2.06
37	0.12	2.19
39	0.12	2.31
41	0.12	2.43
43**	0.12	2.55

*At Day 19, the pots amended with *P. sylvestris* litter extracts was accidentally first amended with litter extract of *E. hermaphroditum*, and the pots supposed to be amended with *E. hermaphroditum* litter extract were instead watered with de-ionized water.

** Days of N fixation measurement of *P. schreberi*.

procedure. At the end of the experiment a total of 2.55 g of litter from each species had been extracted by 1100 ml of water for each pot. These amounts equate to approximately 1.5 years of litter fall, extracted in 1 year of average precipitation, on an aerial basis (Wardle, personal communication). Nitrogen fixation rates of *P. schreberi* shoots were determined after 11 and 22 extract additions (21 and 43 days since initiation of treatment application, respectively). Control pots were watered with the same amount of de-ionized water as the litter extract additions at all occasions of treatment.

Nitrogen fixation analysis

Nitrogen fixation rates were estimated using a calibrated acetylene reduction technique (Schöllhorn and Burris 1967). Ten moss shoots were randomly removed from each pot and placed into a 22-ml glass culture tube. The tubes were fitted with a septum and 10% of the total headspace was evacuated and replaced with acetylene. The tubes were incubated for 24 h before analysed for total ethylene production on a Perkin Elmer, Clarus 500 GC and Turbomatrix 40 headspace injector. The samples were injected isothermally 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. These estimates were then converted to ethylene mass ($\mu\text{mol g}^{-1} \text{day}^{-1}$) using the universal gas law ($PV=nRT$). The data are thus presented as acetylene reduced, which is closely correlated with N fixation. DeLuca *et al.* (2002a) described that the *Pleurozium-Nostoc* association and other *Pleurozium-cyanobacteria* associations reduce acetylene at a ratio of 3 mol of ethylene per mol N. To know the dry weight of the moss, all samples were dried at 65°C for 48 h before weighing and

calculating the acetylene reduction rate ($\mu\text{mol g}^{-1} \text{day}^{-1}$). This was done so that N fixation could be reported on a dry weight basis.

Statistical analysis

In both experiments, the five sub-replicates that were placed into separate blocks were averaged, to create a single datum. This created a sample size of three, for all litter x stand age and water x stand age treatment combinations. Data were first evaluated for assumptions of normality and homogeneity of variance, and were found to reasonably meet these assumptions. For both experiments we evaluated the data using a 2-factor analysis of variance (ANOVA) in the general linear model in SPSS (version 12.0). In the watering experiment, stand age (old or young) and watering treatment were entered as fixed factors. In the watering experiment, where significant differences were detected, a post-hoc one-way ANOVA was conducted to determine treatment differences within young and old stands.

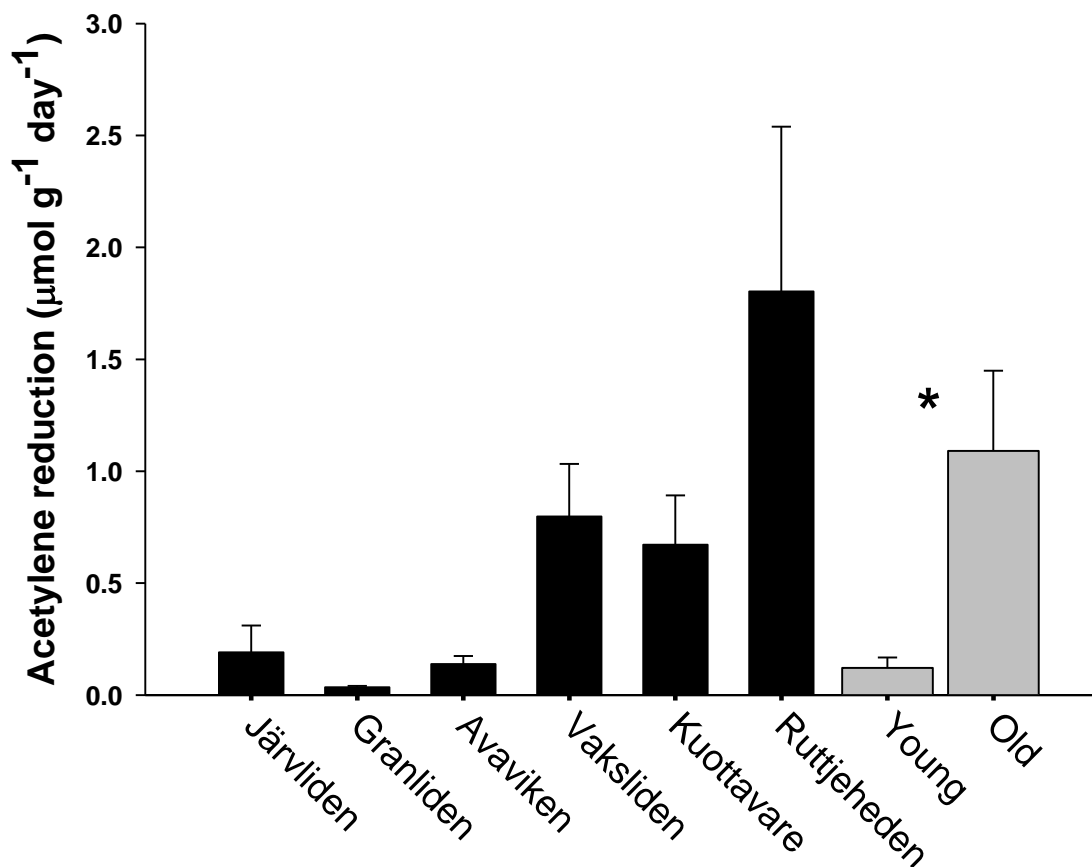


Figure 1. Mean (\pm SE) acetylene reduction rates of *P. schreberi* ($\mu\text{mol g dw moss}^{-1} \text{day}^{-1}$) collected from six boreal forest stands before the start of experimental treatments. A Student's t-test was made comparing N fixation rate between young and old study sites. The * indicates statistically significant differences at $\alpha = 0.01$ ($p = 0.055$, $t = 2.686$, $df = 4$).

RESULTS

Pre-treatment measurements of N fixation rates in *P. schreberi* shoots collected from the six forest stands showed that N fixation was much higher in moss collected from the three old sites (age > 290 years) compared to that of moss collected from the three more recent burned sites (Figure 1). There was also substantial variation of N fixation within stands, especially for the oldest successional site Ruttjeheden.

Water experiment

Addition of water significantly stimulated N fixation rates of mosses (Figure 2), although this was not apparent after 13 days of treatments (Figure 2a). After 45 days of treatment, significant differences emerged, and the most frequent water addition resulted in significantly higher N fixation in old stands, but mosses collected from young stands responded less to any of the water additions (Figure 2b). Throughout the duration of the experiment, all mosses watered demonstrated a small increase in N fixation rate (Figure 2).

Litter extract experiment

There were no significant effects of litter extracts on N fixation of *P. schreberi* 21 days and 45 days after treatment initiation (Figure 3a). However, one litter type with a relatively high nutrient content, *V. myrtillus* appeared to reduce N fixation rates relative to that of the other treatments, but this effect was not statistically significant. As in the watering experiment, stand age significantly determined N fixation rates between stands, with significantly lower N fixation rates in moss collected from young stands relative to old successional stands, throughout the duration of the experiment. Mosses from young stands did not respond significantly differently to that of mosses from old stand to the addition of litter extract.

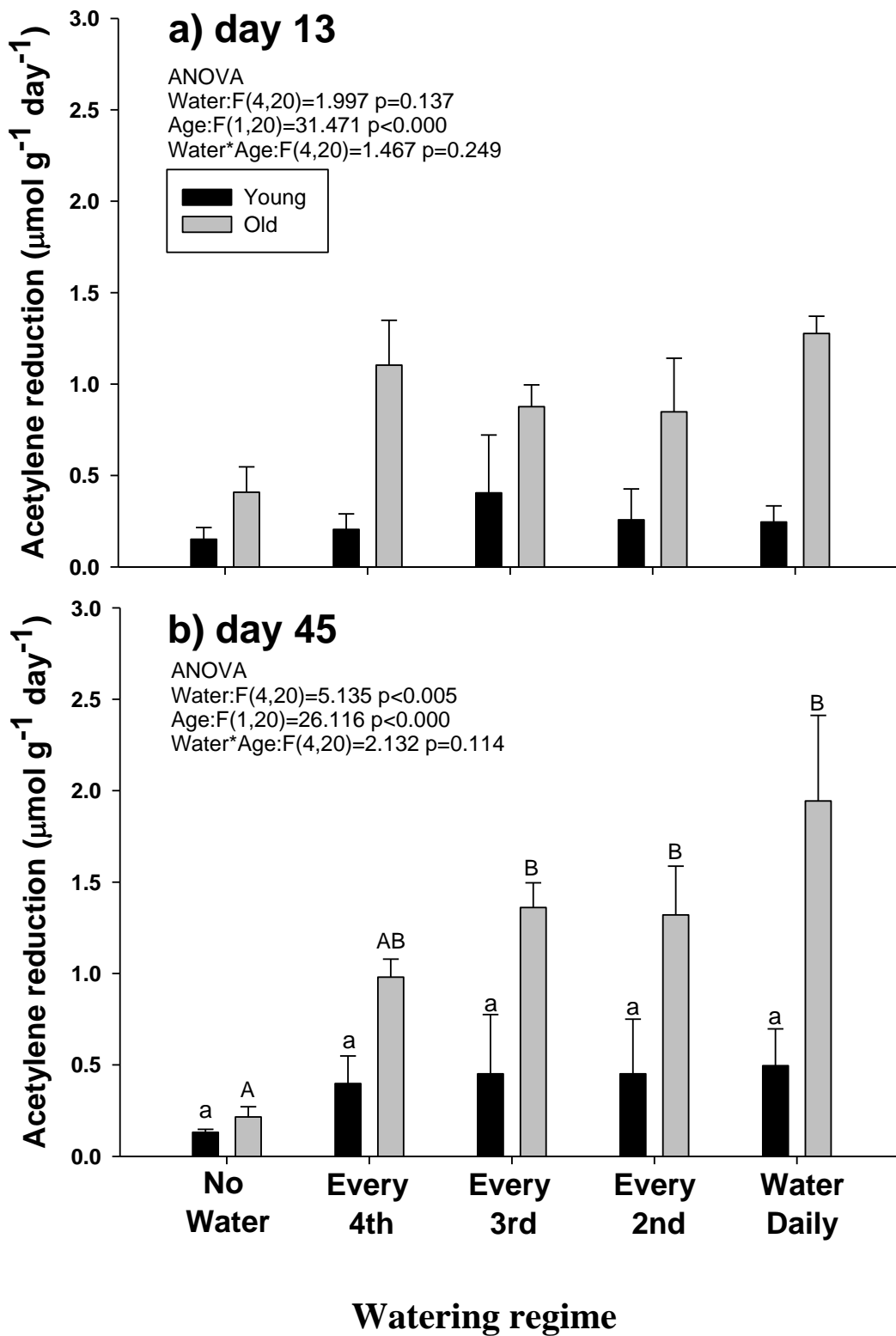


Figure 2. Mean (\pm SE) acetylene reduction rates ($\mu\text{mol g}^{-1} \text{day}^{-1}$) of *P. schreberi* collected from 3 old and 3 young forest sites at (a) 13 days and, (b) 45 days after the start of watering treatments. Letters above the bars (within age groups) denote significant differences between treatments at $p \leq 0.05$.

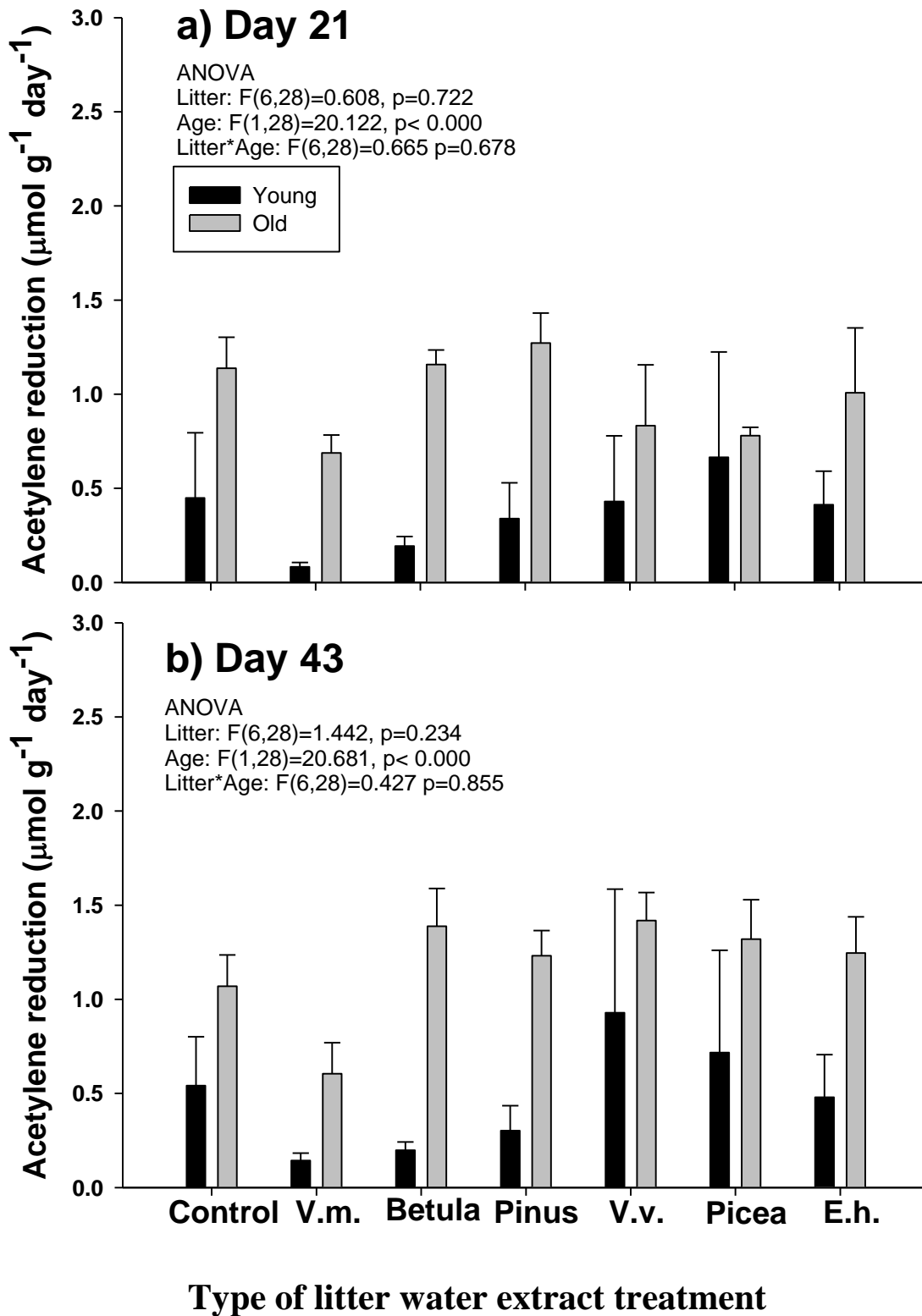


Figure 3. Mean (\pm SE) acetylene reduction rates ($\mu\text{mol g}^{-1} \text{day}^{-1}$) of *P. schreberi* at (a) 21 days and, (b) 43 days following repeated application of litter extracts to mosses from young and old sites. The different treatments were: de-ionized water (Control), *Vaccinium myrtillus* (V.m) *Betula pubescens* and *Betula pendula* (Betula), *Pinus sylvestris* (Pinus), *Vaccinium vitis-idaea* (V.v), *Picea abies* (Picea) and *Empetrum hermaphroditum* (E.h).

DISCUSSION

It is poorly understood what factors influence N fixation within forest, and what drives variability in these patterns across the landscape. In this study I identified two significant factors that are generating variability in N fixation, and these include moisture availability and stand age. My data also suggests that the direct effect of litter is likely minor compared to stand age and water status.

Stand age has previously been identified as a factor affecting the N fixation rate in the association between *P. schreberi* and cyanobacteria (Zackrisson *et al.* 2004). The mechanisms for the increase in N fixation with time since fire is not fully understood, but it can be due to different factors such as vegetation, nutrient availability and moisture that co-vary as plant succession proceeds. After a fire, the plant community change from domination of early successional species like grasses, deciduous trees, *P. sylvestris*, and *V. myrtillus* with relatively high foliar N content towards domination of mainly ever-green old successional species like *E. hermaphroditum* and *P. abies* with lower foliar N content. Such differences in vegetation composition along successional fire gradients might have important direct and indirect effects on N fixation patterns in boreal forests. In this study, where only a direct effect was tested, no significant influence of litter was detected. However, significant effects of these species may occur in other ways not tested in this experiment. For instance, there is likely great variation in the nutrient content of the litters used, which may only become available and influence mosses and cyanobacteria during decomposition.

Although there was no statistically significant effect of the litter extracts added, it appeared that some variation in N fixation rate in response to plant litter extracts was beginning to emerge at the end of the experiment, and some of this variation appeared to be related to variation in litter chemistry between the species. The cyanobacteria living on *P. schreberi* have the capacity to obtain the carbon they need, through photosynthesis, but it is fully plausible that simple sugars leached from the litter may serve as a carbon source for the cyanobacteria and thereby enhance N fixation. In a study by Steinberg and Meeks (1991), it was found that symbiotic cyanobacteria on moss also use carbon from their host. However, it is not known if they may also utilize carbon from litter throughfall. If they are able to use some easily available carbon from litter, then variation in litter quality among species could be an important factor driving N fixation rates. Likewise, the small increase in fixation in response to *V. vitis-idaea* could be due to specific phenolic substances present in extracts that complex N, and thereby reduce the available amount of N and enhance the demand for N fixation.

The decrease in N fixation for mosses watered with extracts of *V. myrtillus* could be due to easily leachable N compounds that inhibited N fixation. It is generally known that excess of N in the environment reduces biological N fixation (Chapin *et al.* 1991). In a study by Wardle *et al.* (2003) it was found that early successional species, such as *V. myrtillus* and *B. pendula*, contain a higher proportion of water soluble N than the other dominant boreal species investigated. Thus the observed decrease in N fixation with extract addition of *V. myrtillus* could be due to a fertilization effect.

Many boreal plant species including *V. myrtillus* are also known to produce high amounts of C based allelochemicals in their leaves (Witzell and Shevtsova 2004), and it could also be so that these may exert negative (allelopathic) effects on N fixing

cyanobacteria. For example, Schimel *et al.* (1988) reported in a study from the Alaskan taiga that tannins from *Populus balsamifera* inhibited N fixation in nodules of *Alnus tenuifolia*. In a study by Kapustka and Rice (1976) it was also shown that some phenolic acids found in soil inhibited growth of N fixing bacteria. Whether this is true for *V. myrtillus* remain to be investigated. Further, it is also possible that differences in N fixation are not due to a direct effect on the cyanobacteria, but rather an indirect effect on the mosses. During the experiment, a noticeable colour change occurred in mosses treated with *V. myrtillus* and *Betula sp.* extracts (data not shown), which both demonstrated the lowest N fixation rates among the treatments. Thus, it is possible that the decrease in N fixation was due to reduced fitness of the moss host in supporting its cyanobacterial partner. While a significant litter effect was not detected within the duration of my experiment, the above outlined mechanisms by which different litters may influence N fixation may require a longer period of time for significant differences to emerge.

Another factor responsible for the observed N fixation pattern along fire choronsequences in boreal forests (Zackrisson *et al.* 2004, Lagerström *et al.* 2007) could be related to increased moisture conditions with increasing time since fire. Moisture in the humus layer increases with time since fire (Table 3) and is positively correlated to N fixation rates (Fig 1). The higher moisture content of humus in older sites can be due to a build up of humus containing many capillary spaces that retain water at very low water potentials. My results from the laboratory experiment confirm that access to water is important for the N fixation process as N fixation was generally found to increase with increasing frequency of water added to *P. schreberi*. Other studies from arctic tundra (Svalbard, Norway) have also shown that N fixation in moss associated cyanobacteria varies depending on the water content (Zielke *et al.* 2005). Solheim *et al.* (2002) found that increasing precipitation enhanced the N fixation rate in the association between *Hylocomium splendens* and cyanobacteria in a subarctic forest in Abisko, Swedish Lapland. Generally moisture availability has been found to be an important factor for N fixation (Turetsky 2003). One possible mechanism that affects N fixation can be that water reduces the metabolic processes in the cyanobacteria with increasing negative water potentials (Hartley and Schlesinger 2002). This could include a variety of cell functions including photosynthesis, membrane transport, and nitrogenase production and activity. Water can also be important to enhance the transport of carbon or other important elements, such as P, Fe, Mo or Ni to the bacteria.

Interestingly, the effect of water additions was strongest in *P. schreberi* collected from old successional stands. Water had surprisingly weak positive effects on moss from young successional stands. This different response could be due to an initial higher density of N fixing cyanobacteria on the moss surface of *P. schreberi* in old successional forests as reported by DeLuca *et al.* (2007). If mosses from old successional stands were populated by a higher density of bacteria, it would be likely that they were able to show a stronger response to the water treatment than less inhabited moss (e.g. moss from young successional stands). An overall high moisture content in mosses in old successional stands might support a larger population of bacteria. This may also help to explain the higher fixation rate found in old successional stands.

One important difference between the water and litter extract experiments is that litter treatments had a small non significant effect in both young and old sites, whereas water

treatments only seemed to effect N fixation in old sites. This might suggest that some characteristic of these litter extracts influences a process that regulates N fixation across the entire chronosequence, whereas water interacts with some regulating factor that is only present in old successional stands.

Conclusion

In this study I have found that the N fixation by cyanobacteria living on *P. schreberi* is influenced by both moisture (water availability) and stand age. My experiments suggest that N fixation is a sensitive process that can vary as a result of a combination of interacting factors. Changes in climate (precipitation regimes) will certainly have an impact on the N fixation pattern. It is also possible that the way that forest vegetation changes following forest management will impact a change the N fixation magnitude. This study advances our knowledge of some of the factors that drive variation in N fixation in boreal ecosystems, but to understand how different changes will affect N fixation in different scenarios for the future, further studies are needed.

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